Distribution and Habitat Association of Western Chicken Turtles (*Deirochelys reticularia miaria*) in Texas

Final Report

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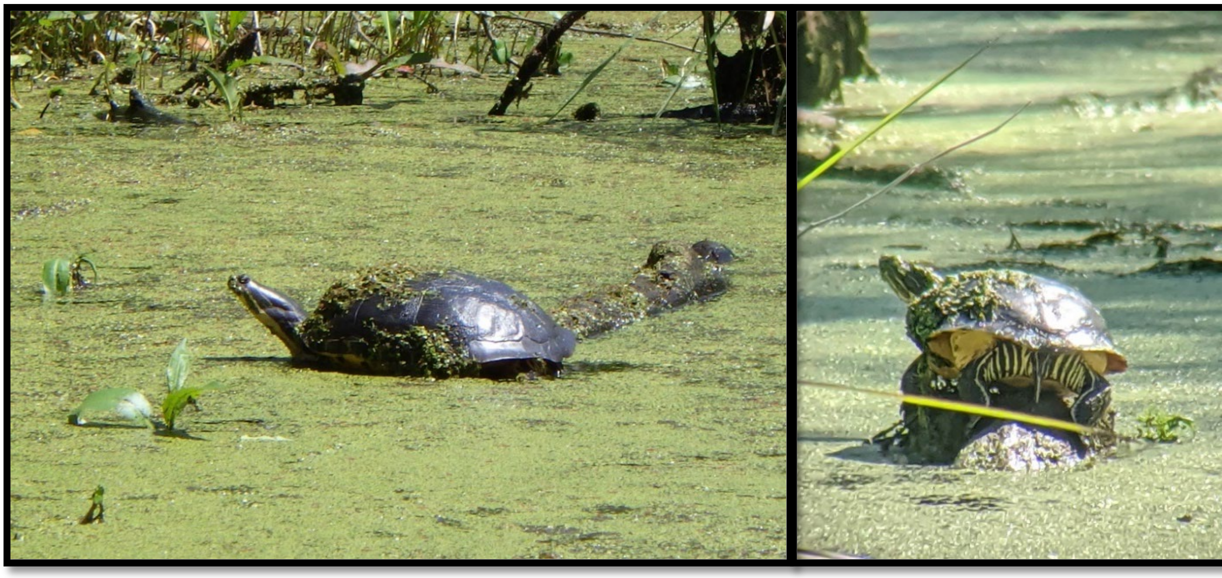

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List of Abbreviations

AGFC	Arkansas Game and Fish Commission
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
AUC	Area under the curve
BAVS	Binocular assisted visual surveys
CN	Cellulose nitrate
CON	Control (in reference to site IDs)
Conf. level	Confidence levels
COVID-19	2019 novel coronavirus (SARS-COV-2)
CPUE	Catch per unit effort
CSE	College of Science and Engineering
CSS	Canid scent surveys
DAPTF	Declining Amphibian Task Force
DEM	Digital elevation model
DI	De-ionized
DT	Distance traveled
eDNA	Environmental DNA
EIH	Environmental Institute of Houston
FAA	Federal Aviation Administration
FAV	Floating aquatic vegetation
GBIF	Global Biodiversity Information Facility
GLM	Generalized linear model
GoF	Goodness-of-Fit
GPS	Global Positioning System
HA	Historic accounts (in reference to site IDs)
HC	Historic counties (in reference to site IDs)
HSG	Hydrologic Soil Groups
IUCN	International Union for Conservation of Nature
Max-SD	Maximum shell depth
Max-SW	Maximum shell width
MDC	Missouri Department of Conservation
Mid-PL	Midline plastron length
Mid-SCL	Midline straight carapace length
MNHP	Mississippi Natural Heritage Program
NC	New counties (in reference to site IDs)
NLCD	National Land Cover Database
NOAA	National Oceanographic and Atmospheric Administration
NRCS	National Resources Conservation Service
NTU	Nephelometric Turbidity Units
NWI	National Wetland Inventory
NWR	National Wildlife Refuge
ODWC	Oklahoma Department of Wildlife Conservation
OPP	Opportunistic (in reference to site IDs)
ORT	Online reporting tool
PAO	Proportion of site occupied

PCR	Polymerase chain reaction
PIC	Pilot in Command
PIT	Passive integrated transponder
PW	Plastron width
QC	Quality control
qPCR	Quantitative polymerase chain reaction
Rel. Abund.	Relative abundance
RS	Road surveys
Drone$_{M2}$	Specific to the Mavic 2 Enterprise Dual drone platform
Drone$_{P4}$	Specific to the Phantom 4 Multispectral drone platform
SAV	Submerged aquatic vegetation
SDM	Species distribution model
SRA	Sabine River Authority; also applies to supplemental site IDs sampled in Year 2
SSA	Species status assessment
SE	Standard error
SWQM	Surface Water Quality Manual
TBC	Tangled Bank Conservation, Inc.
TCEQ	Texas Commission on Environmental Quality
TCPA	Texas Comptroller of Public Accounts
TFTSG	Tortoise and Freshwater Turtle Specialist Group
TNM	The National Map
TPWD	Texas Parks and Wildlife Department
TTWG	Turtle and Tortoise Working Group
TXDOT	Texas Department of Transportation
UHCL	University of Houston–Clear Lake
Unk	Unknown
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WCT	Western Chicken Turtle
WMA	Wildlife Management Area
WS	Walking surveys

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EXECUTIVE SUMMARY

The southeastern U.S. represents one of the most ecologically diverse regions in the world. In recent decades, this area has exhibited declines in wetland-dwelling amphibian and reptile populations. Land alteration has led to fragmentation of formerly expansive contiguous tracts of wetlands while urban and agricultural sprawl have entirely removed wetland habitat in many areas. This may be particularly detrimental to certain turtle species, like the Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*), due to their dependency on and movement between intermittent wetlands. A petition to protect the WCT under the Endangered Species Act has been submitted to the U.S. Fish and Wildlife Service (USFWS), though currently the WCT does not hold any legal protections in Texas. The current project serves to inform the USFWS Species Status Assessment process via completion of the following objectives: 1) estimation of the current range, distribution, and habitat associations of WCT in Texas; 2) evaluation of the efficacy and efficiency of traditional and novel survey protocols for detecting WCT, and 3) making recommendations of further landscape scale research needs for the species in Texas.

Spatial data for historic occurrences of WCT and wetland boundary data from the National Wetland Inventory were used to generate randomized locations within boundaries of potential habitats. In addition to randomized site locations, non-randomized locations were selected based on areas of known occupancy, areas identified as potential habitat throughout the study period, and through supplemental funding provided by the Sabine River Authority of Texas. Sample periods were split in to in-season (March-July of 2020-2022) and out-of-season (August 2021-February 2022). A combination of protocols were used to assess habitat associations and test efficacy and efficiency for detection of WCT including: five types of environmental DNA (eDNA) samples (A-0.45, A-3.0, R-0.45, R-3.0, and soil samples), binocular assisted visual surveys (BAVS), walking surveys (WS), drone surveys with two imagery types (M2 and P4), hoop trap surveys, game camera (GC) surveys, canid scent surveys (CSS), and road surveys (RS). In addition to field surveys, a citizen-science based online reporting tool (ORT) was developed to compile reports of WCT throughout their range. Habitat associations at small- and landscape-scales were conducted for locations resulting in WCT detections versus those not resulting in detections. Additionally, a protocol comparison rubric was developed to make best-recommendations toward efficacy and efficiency of protocols for future studies. The rubric was developed to consider broad categorical concerns (logistics, statistics, and costs) with multiple sub-categories that were ranked based on results of each protocol applied in the study. The rubric was tested using four potential scenarios for future survey goals and/or objectives.

From March 2020 through July 2022, 66 sites in 33 counties were sampled during the in-season period resulting in 346 sampling events. Four sites in four counties were sampled monthly between August 2021 through February 2022 during the out-of-season period resulting in 28 sampling events. A total of 102 WCT detections were made over the course of the study. In-season efforts resulted in 88 confirmed detections of WCT across all events, sites, and protocols while out-of-season efforts resulted in 14 confirmed detections of WCT across all events, sites, and protocols. Across all protocols (including the ORT), WCT presence was confirmed at 12 locations. At locations with confirmed detections, WCT activity was documented in all calendar months except January and November. Across all years, six WCT were physically captured at five sites representing four counties.

Overall, sites with a designated wetland classification or observed wetland type of Freshwater Emergent or Freshwater Pond had the highest WCT detections. The probability of detecting

WCT was positively correlated with decreasing Secchi depth and negatively correlated with increasing specific conductivity. Canopy cover in the middle height category (0.5-5 m) was lower while canopy cover in the lower height category (< 0.5 m) was higher during events where WCT were detected. Additionally, detections of WCT were increased during events with dominant ground cover categories including in-water vegetation (submerged aquatic vegetation or floating aquatic vegetation) compared to sites with a dominant ground cover type consisting of bareground/duff or grasses/herbs. A combination of wetland classification, observed wetland type at site, Secchi depth, middle and lower height canopy covers, and dominant ground cover type was the best predictor of WCT detection.

In the historic SDM, raw land cover and road density co-variates contained the most useful information. Conversely, the current SDM predicted high habitat suitability in areas not directly associated with city centers, indicating that city centers and major highways were predicted to have the least suitable habitat for WCT with the most suitable habitat occurring around urban fringes. In the current SDM, the majority of predicted habitat resided in the southeastern coastal plain and in low-lying areas of major river basins in central- and northeast Texas. Visual comparison of the presence and current SDMs show convergence in areas where more suitable habitat was predicted overall. We believe that this model represents the most current prediction for WCT distribution, especially considering convergence of the model with the presence SDM.

Overall, protocol detection probability was highest during the in-season sampling period (March-July) and effort as a co-variate was a better predictor than event (as a factor of time). Though we were successful in detecting WCT using 13 of the 14 protocols applied in the current study, some protocols were more efficient and effective than others. Of the protocols with multiple application types (eDNA and drone), A-3.0 and R-3.0 showed the greatest positive deviation from the mean when compared to other eDNA protocols while the M2 showed greater positive deviation from the mean when compared to P4. While our efforts resulted in multiple detections of WCT, total number of detections for each protocol were low. Total number of detections varied between protocols and the proportion of detections did not exceed 25% for any given protocol. Additionally, protocols with the highest detection proportion varied in calculated "catch" per unit effort. For detection of WCT, we recommend A-3.0 and R-3.0 protocols but for capture of WCT, we recommend hoop trap surveys. Ultimately, final protocol selection will depend on the specific question of future surveys, as demonstrated in applications of hypothetical scenarios to our protocol comparison rubric.

Further analysis of small-scale habitat preferences of WCT, their relation to macro-scale ecological factors, and how anthropogenic factors may threaten the availability of each are needed in areas not covered by the current study. In addition to the environmental co-variates we assessed in SDM, future SDM for the WCT should focus on inclusion of other co-variates to the model(s). Though we can make best recommendations for which protocols to apply in a given scenario, our primary recommendation is to apply a combination of sampling techniques, regardless of question or over-arching goal, in order to maximize efficiency and effectiveness for assessment of this cryptic and wide-spread species in Texas. Additional conservation considerations including level of disturbance or destruction to the habitat, stress or risk of injury to the target species or by-catch, and potential for introduction of invasive species or zoonotic diseases are key considerations as future studies aim to assess the habitat associations, distribution, population dynamics, and more for this cryptic, wetland dwelling species.

INTRODUCTION

The southeastern United States represents one of the most ecologically diverse regions in the world (Stein, 2002). In recent decades, this area has exhibited declines in wetland dwelling amphibian and reptile populations, primarily due to habitat loss from agricultural or urban development and commercial international export (Gibbons et al., 2000; Semlitsch and Bodie, 2003; Ceballos and Fitzgerald, 2004; Prestridge et al., 2011; Quesnelle et al., 2015; USFWS, 2016). Land alteration has led to fragmentation of formerly expansive contiguous tracts of wetlands while urban and agricultural sprawl have entirely removed wetland habitat in many areas. This may be particularly detrimental to certain turtle populations due to their dependency on, and movement between, intermittent wetlands (Ryberg et al., 2017; Chyn et al., 2020).

The genus *Deirochelys* (Agassiz 1857) is monotypic and includes the Chicken Turtle (*D. reticularia* [Latrielle 1801]) (Schwartz, 1956; Walker and Avise, 1998; Ewert et al., 2006; Ernst and Lovich, 2009). In the mid-20th century, *D. reticularia* was revised into the three currently recognized subspecies: Eastern (*D. r. reticularia*), Florida (*D. r. chrysea*), and Western (WCT; *D. r. miaria*). General life history trends, population structure, and habitat use are best understood for the eastern subspecies (Gibbons, 1970; Gibbons and Greene, 1978; Demuth and Buhlmann, 1997; Buhlmann, 2008; Buhlmann et al., 2009). In general, Chicken Turtles exhibit a short life span, generally fast growth rates, and can occur as widespread populations (Gibbons, 1969, 1987; Trauth et al., 2004; Ewert et al., 2006; Ernst and Lovich, 2009). These traits are important when assessing threats to the species status as they may artificially increase the perception of rarity and localized habitat alterations may have a greater impact to local populations (Buhlmann, 1995; Dinkelacker and Hilzinger, 2009). Previous investigations have helped close knowledge gaps in WCT population trends, life history traits, and movement patterns, though few studies assess populations on a broad scale (Carr and Tolson, 2017; Ligon et al., 2017; Bowers et al., 2021, 2022a). More recently, studies in Mississippi, Arkansas, and Texas have been unable to detect WCT in areas where the species is anticipated, or have encountered lower than expected detection rates (Dinkelacker and Hilzinger, 2014; Ryberg et al., 2017; Jones 2022; McKnight et al., 2022).

The WCT differs from their eastern conspecifics in a variety of ways. The Eastern and Florida subspecies rely on a primarily carnivorous diet while WCT appear to be more omnivorous (Jackson, 1996; Demuth and Buhlmann, 1997; McKnight et al., 2015a; Ryberg et al., 2017). Furthermore, the Eastern and Florida subspecies exhibit an atypical reproductive strategy by nesting in the late fall and early spring, exhibiting egg retention over winter months, and producing multiple clutches in a season (Cagle and Tihen, 1948; David, 1975; Buhlmann et al., 1995). Conversely, the WCT exhibits a discrete nesting season similar to other aquatic turtles, with nesting occurring up to three times during the late spring to summer months (April-July) (McKnight et al., 2015b, 2018; Carr and Tolson, 2017; Bowers et al., 2022b). Additionally, WCT are believed to demonstrate earlier onset of prolonged aestivation (mid-July to February) compared to their eastern counterparts where aestivation periods are limited or non-existent (Gibbons, 1969; Gibbons and Greene, 1978; McKnight et al., 2015b; Bowers et al., 2021).

The WCT's range extends west of the Mississippi River into Louisiana, Arkansas, Missouri, Oklahoma, and Texas (Ewert et al., 2006; Buhlmann et al., 2008; Ernst and Lovich, 2009; TTWG, 2017). It has been suggested that the Guadalupe River serves as the southwest-most boundary of the species range (Ryberg et al., 2016, 2017). In Texas, the WCT's historic range extends across 79 counties with public reports dating back to 1931 (Figure 1) (Dixon, 2013;

USFWS, 2016; Ryberg et al., 2016, 2017; Franklin et al. 2019; Bowers et al., 2021). The species is known to prefer ephemeral wetlands adjacent to or within prairie habitats (Buhlmann, 1995; Buhlmann et al., 2008; Ernst and Lovich, 2009). Recently, it has been suggested that the species may exhibit partial or irruptive nomadism due to multiple movements and/or return(s) to spatially distant wetland habitats (Bowers et al., 2021).

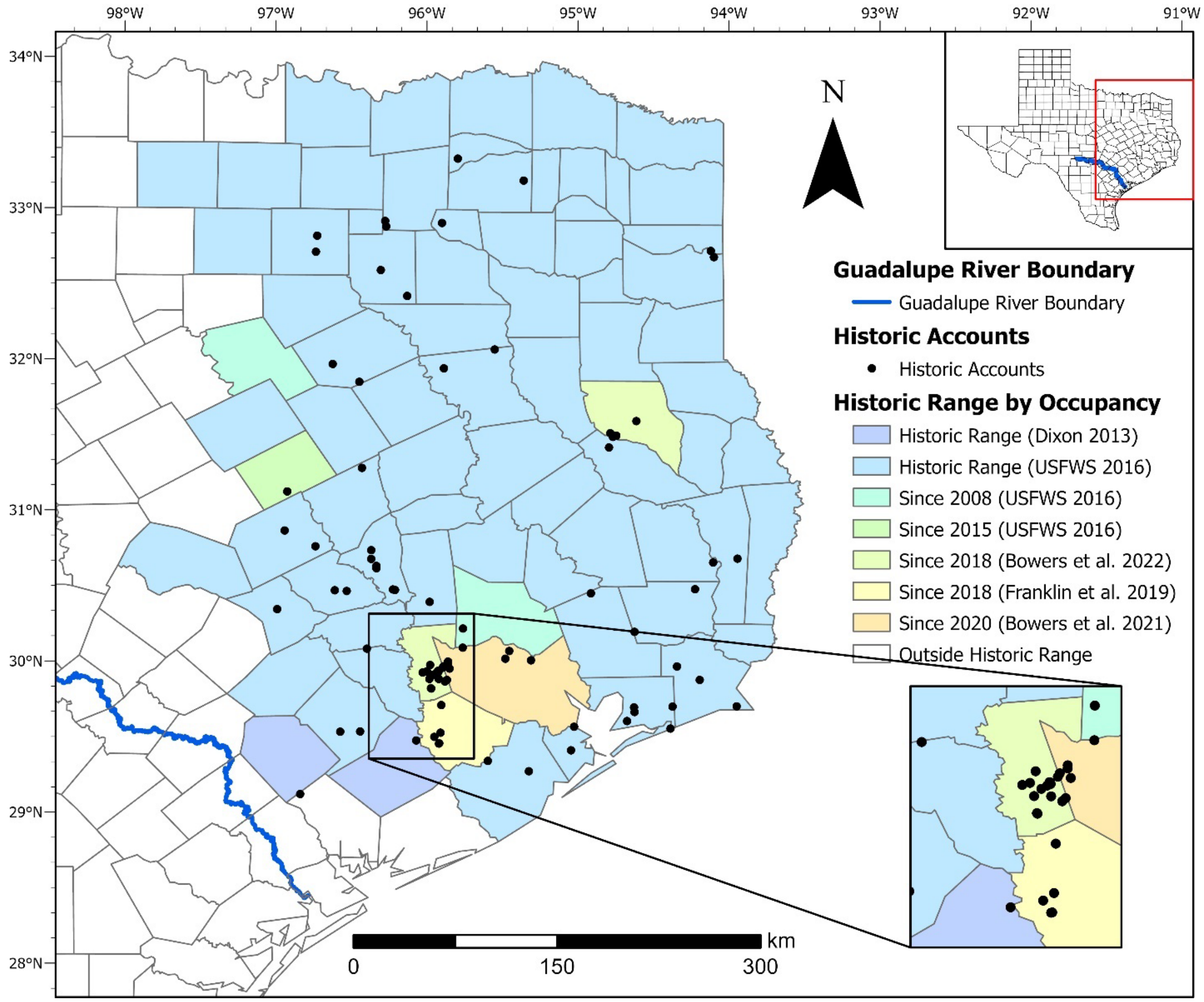


Figure 1 Historic range (*N* = 79 counties) and accounts (*N* = 91 accounts) of Western Chicken Turtles in Texas. Range from Dixon (2013), USFWS (2016), Franklin et al. (2019), and Bowers et al. (2021, 2022a). County colors correlate to age of last established occupancy, e.g., cooler colors equate to older known counties of occupancy while warmer colors equate to counties with more recently established or confirmed occupancy. Historic accounts extracted from Adams and Saenz (2011; *n* = 1), Ryberg et al. (2017; *n* = 3), Franklin et al. (2019; *n* = 1), VertNet (2020; *n* = 50), research grade reports from iNaturalist (2020; *n* = 34), and Bowers et al. (2021; *n* = 2). Some iNaturalist coordinates are approximate (obscured when reported, *n* = 13).

A recent population assessment in Texas reviewed threats to WCT populations with urban expansion identified as the greatest current and future threat to the species (Ryberg et al., 2017). The study identified "hot spots" of potential population locations based on historic ranges and records, however, after exhaustive efforts, few individuals were observed (*n* = 3). The project team attributed difficulty in locating individuals to the species' exhibition of discrete seasonal activity patterns and potential sampling method bias, recommending that a combination of sampling methods be used to reduce bias in future surveys (Dinkelacker and Hilzinger, 2014; McKnight et al., 2015c; Ryberg et al. 2016).

Traditional aquatic turtle sampling techniques, such as hoop traps, have been shown to exhibit an inherent bias towards certain species and age or size classes within a population (Ream and Ream, 1966; Frazer et al., 1990; Gamble, 2006). In general, trapping requires excessive effort, physically and financially, to be conducted properly and causes varying degrees of habitat disturbance (Gibbons, 1969; Gibbons and Greene, 1978; Morton et al., 1988; McKnight et al., 2015c; Welbourne et al., 2015; Ryberg et al., 2017). Though traditional methods are recommended for population studies (especially demographic assessments), other novel detection methods have emerged including use of environmental DNA (eDNA), drones, remote camera sensing, and trained detector dogs. These techniques are effective in detection of species, less invasive than traditional methods, can cover a broader geographic scope, and are ultimately more cost-effective (Koh and Wich, 2012; Anderson and Gaston, 2013; Stein et al., 2014; Schofield et al., 2017; Daniels, 2018; Rees et al., 2018).

Collection of eDNA has been widely used to detect presence of cryptic or hard-to-find species (Jerde et al., 2011; Wilcox et al., 2013; Sigsgaard et al., 2015; Thomsen and Willerslev, 2015; Barnes and Turner, 2016; Lacoursière-Roussel et al., 2016; Nevers et al., 2018). Due to the non-lethal and minimally invasive nature of this method, it is rapidly becoming a preferred method for detection of species of concern (Thomsen et al., 2012; Raemy and Ursenbacher, 2018; Matthias et al., 2021). Within the last decade, use of drones has become popular for assessing and monitoring habitat use and animal behavior (Vermeulen et al., 2013; Wilson et al., 2017; Biserkov and Lukanov, 2017; Corcoran et al., 2019; Driggers et al., 2019; Davis et al., 2020). As this technology has grown, researchers have discovered that drones provide a safer and less disturbing approach to monitor cryptic or flighty species (Christie et al., 2016; Vallery, 2018). Remote camera sensing ("camera traps") has been used globally to document activities of difficult to observe species (Silveira et al., 2003; Kelly, 2008; Ridout and Linkie, 2009; Royle and Gardner, 2011). Camera traps are effective in recording behaviors and activities at times when they may not be typically observed by humans (Doody and Georges, 2000; Trolle and Kéry, 2005; Welbourne, 2013; Baxter, 2017). Finally, use of trained detector dogs has been successful in searches for cryptic or hard to find species for over 20 years (Weldon and Fagre, 1989; Cablk and Heaton, 2006; Cablk et al., 2008; Nussear et al., 2008; Hoffman, 2014; Roda et al., 2021). Recently, researchers have shown an interest in use of detector dogs, especially regarding turtle and tortoise surveys (Witherington et al., 2017; Powers, 2018; Richards, 2018; Jean-Marie et al., 2019; Harris et al., 2020; Statham et al., 2020). Using this suite of novel search methods in combination with more traditional aquatic turtle sampling techniques should maximize the likelihood of detection of cryptic species, such as the WCT.

Deirochelys reticularia holds an International Union for Conservation of Nature (IUCN) Red List status of "not evaluated", but in 2011, the species was provisionally designated as "Near Threatened" by the IUCN Tortoise and Freshwater Turtle Specialist Group (TFTSG) and currently maintains that designation on the TFTSG Red List (Carr and Tolson, 2017; TTWG, 2017; Rhodin et al., 2018). Currently, the Chicken Turtle is Critically Imperiled in Missouri, Imperiled in Arkansas and Louisiana, Vulnerable in Mississippi (though overall status is unknown), and a Species of Greatest Conservation Need (SGCN) in Oklahoma and Texas (AGFC, 2005; Holcomb et al., 2015; ODWC, 2016; MNHP, 2018; TPWD, 2020; McKnight et al., 2022; MDC 2022).

A petition to include the WCT as Threatened under the Endangered Species Act has been submitted to the U.S. Fish and Wildlife Service (USFWS, 2011), which is under review. As part of the review process, the USFWS has been tasked with developing a Species Status Assessment (SSA) for the WCT, including updated and current information for habitat associations and distribution throughout its current range. The present study intends to inform the SSA process via the following goals:

1. address the status of the WCT in Texas, and
2. inform future landscape-level research on WCT by establishing best survey methodologies and estimating range, distribution, and habitat associations.

We aim to address these goals via a series of tasks, including:

(Task 1) conducting surveys utilizing various methods at sites representative of all habitat types within the modeled species range in Texas,
(Task 2) estimate the current range and distribution of and determine habitat associations for the WCT, and
(Task 3) evaluate efficacy and efficiency of various survey methods for WCT

Through these goals and tasks, we are able to provide recommendations for future landscape scale research needs for the species in Texas. The Environmental Institute of Houston (EIH) has worked with state and federal conservation and resource managers to ensure the study design meets the needs of the SSA process. Data from this study may be used to support future conservation and management decisions for the species.

METHODS

Survey Site Selection

Survey locations were grouped into six categories: Historic Account, Historic County, New County, Control, Opportunistic, and Supplemental (Table 1). Locations within the Historic Account, Historic County, and New County categories were randomly generated using ArcGIS Pro (ESRI, 2021a) while Control, Opportunistic, and Supplemental locations were non-randomly selected. Table 1 provides a description of selection criteria and the following provides a detailed description of the randomized location generation and non-randomized site selection process.

Table 1 Field survey location category descriptions for Western Chicken Turtle (WCT) surveys in East Texas. Abbreviations in parentheses represent corresponding site ID labels.

Location Category	Description
Historic Account (HA)	Randomly generated locations restricted to a 5-km radius circular buffer around historic account locations.
Historic County (HC)	Randomly generated locations within counties containing historic accounts, but outside of the 5-km radius circular buffer around historic account locations.
New County (NC)	Randomly selected locations within counties in the WCTs historic range but lacking specific spatial historic account location(s).
Control (CON)	Non-randomly selected locations occurring in areas with confirmed WCT presence. Sampling was conducted in coordination with agencies conducting ongoing research.
Opportunistic (OPP)	Non-randomly selected locations identified during the current study. Generally coincident with new reports of WCT observations, detections made during this study, or land access granted in areas of likely WCT occupancy.
Supplemental (SRA)	Locations selected as part of a supplemental study funded by the Sabine River Authority of Texas (SRA) (Gordon et al., 2021a; Appendix A).

Randomized Survey Location Generation

Spatial data for historic occurrences of WCT were compiled from VertNet (accessed 20 January 2020), iNaturalist (accessed 20 February 2020), and existing literature (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019) (n = 89). VertNet data were extracted using the search term "Genus=*Deirochelys*"; we did not search for misspellings. VertNet data were filtered for records in Texas including GPS coordinates or descriptions specific enough to be georeferenced (e.g., "3.25 miles southeast of Dallas at the IH-45 bridge crossing over the Trinity River"). iNaturalist data were extracted using the following search criteria: 1) "Species=*Deirochelys*", 2) "Location=Texas", and 3) the "Research Grade" filter activated. Some iNaturalist records were previously obscured, so locations are approximate. Ryberg et al. (2017) did not report specific coordinates, so spatial data were georeferenced in ArcGIS Pro using a raster overlay. A full list of historic accounts used for randomized survey location selection is provided in Appendix B.

Wetland boundary data from the National Wetland Inventory (NWI; USFWS, 2019) were used to generate randomized GPS coordinates. Polygons of major wetland classifications (marine and estuarine, freshwater pond, freshwater emergent, freshwater forested/shrub, lake, and riverine) were overlaid with historic account locations. Though WCT are thought to have an affinity for stagnant, shallow, seasonally fluctuating wetlands (Buhlmann et al., 2009; Bowers et al., 2021), Riverine and Estuarine habitats were included based on historic account location(s) and presence of these habitat types in previous habitat proximity analyses (Ryberg et al., 2016; Table 2). For each major wetland classification type, a target number of base sites (described below) was calculated using this weighted distribution.

Table 2 Weighted distribution used for randomized survey location generation for each major National Wetland Inventory (NWI) classification (USFWS, 2019). Relative composition of each classification based on previous habitat proximity analyses (Ryberg et al., 2016). The target number of base sites for each field survey location category is provided for each wetland classification.

NWI Wetland Classification	Short Name	Relative Composition	Target # Base Sites
Freshwater Pond	Pond	0.56	8
Freshwater Emergent	Emergent	0.22	4
Freshwater Forested/Shrub	Shrub	0.14	2
Lake	Lake	0.03	1
Riverine	River	0.03	1
Estuarine and Marine	Estuary	0.02	1
	Total	**1.00**	**17**

Eastern Chicken Turtles (*D. r. reticularia*) exhibit disproportionate home ranges between male (6.8 ha [0.068 km^2]) and female (2.8 ha [0.028 km^2]) conspecifics while no differences in home range have been detected between sexes for WCT populations in Texas (Buhlmann, 1995; Bowers et al., 2021). A previous assessment of WCT preferred habitat in Texas showed no difference in habitat preference between 1- and 5-km buffers around historic accounts, but there was a significant shift in habitat type when compared to a 10-km buffer (Ryberg et al., 2016). Additionally, mean total distance traveled for WCT in Texas was 4.1 ± 1.78 km with a maximum observed total distance of 7.0 km (Bowers et al., 2021). In an effort to avoid exclusion of potential habitat, we selected a 5-km radius buffer (78.54 km^2 area) around the historic accounts compiled as part of this study to randomly generate locations. This maximized preferred habitat within the area around historic coordinates and allowed for variation in level of descriptiveness for point location data but did not expand so far outside of the documented home range that a shift in available habitat type could cause for efforts in non-preferable areas.

Generation of Randomized Coordinates

For Historic Account sites, a 5-km radius circular buffer was applied to historic account locations and potential site coordinates were randomly generated within major wetland classification polygons residing within the buffer zone (Figure 2). Historic County sites were generated in counties including historic accounts, but outside of the 5-km buffer zone around historic accounts. To expand sampling effort outside of areas immediately associated with historic accounts, wetland polygons within the 5-km buffer zone were excluded and additional site coordinates were randomly generated in NWI-designated wetland habitats within each county. To further expand sampling efforts outside of counties with historic accounts, wetland polygons were restricted to the remaining counties within the WCTs historic range (USFWS, 2016). New County sites were evenly distributed between wetland classifications within these counties to ensure inclusion of all habitats throughout the species historic range.

Randomized Site List Compilation

Order of site coordinates was further randomized using the “Randomize” function in Microsoft Excel 2016 to determine final sorting hierarchy. Sites were assigned “Base” and “Oversample” classifications to designate priority locations for sampling. The number of Base sites (i.e., primary locations) for each wetland type were weighted based on Table 2. Oversample sites (i.e., back-up locations) were produced for each wetland classification to provide alternate locations should Base site(s) not be accessible. Site IDs were generated to differentiate between Historic Account (“HA”), Historic County (“HC”), and New County (“NC”) locations.

Replacing Dropped Sites

Due to the randomization process, multiple locations were generated within a 5-km radius of Control sites (described below). These coordinates were “dropped” (e.g., removed from the sample design) as “Within 5-km of Control” to further increase spatial distribution of randomized locations (Figure 3). Inevitably, some Base sites were dropped prior to field sampling for various reasons (described in Table 3). In instances where randomized location(s) no longer met the original wetland classification (e.g., wetland was replaced with parking lot), we used aerial imagery from Google Earth Pro to survey within 500 m of the randomized coordinates for suitable habitat matching the original wetland classification. If matching habitat was not available within 500 m (e.g., closest body of water was a pond when the original wetland classification was riverine), the site was dropped as “Non-target”. If site access was denied, the site was dropped as “Access denied”. If, after multiple attempts, the landowner was non-responsive, or we were unable to find specific contact information for a particular set of coordinates, the site was dropped as “Unable to contact landowner”. Finally, if a given set of coordinates was deemed inaccessible, the site was dropped as “Inaccessible”.

To comply with the randomized sampling design, Base sites were replaced with the first available Oversample site matching the Site Category (Column D), Site Type (column E), and Wetland Type (Column G) of the dropped Base site (Figure 4). For example, if Base site HA002 (row 24 in Figure 4) was dropped for a given reason, it was replaced with the first available Oversample site (e.g., HA045, row 32 in Figure 4). This randomized design and dropped site replacement protocol follows similarly to methods used for the National Aquatics Resources Surveys (outlined in USEPA, 2018).

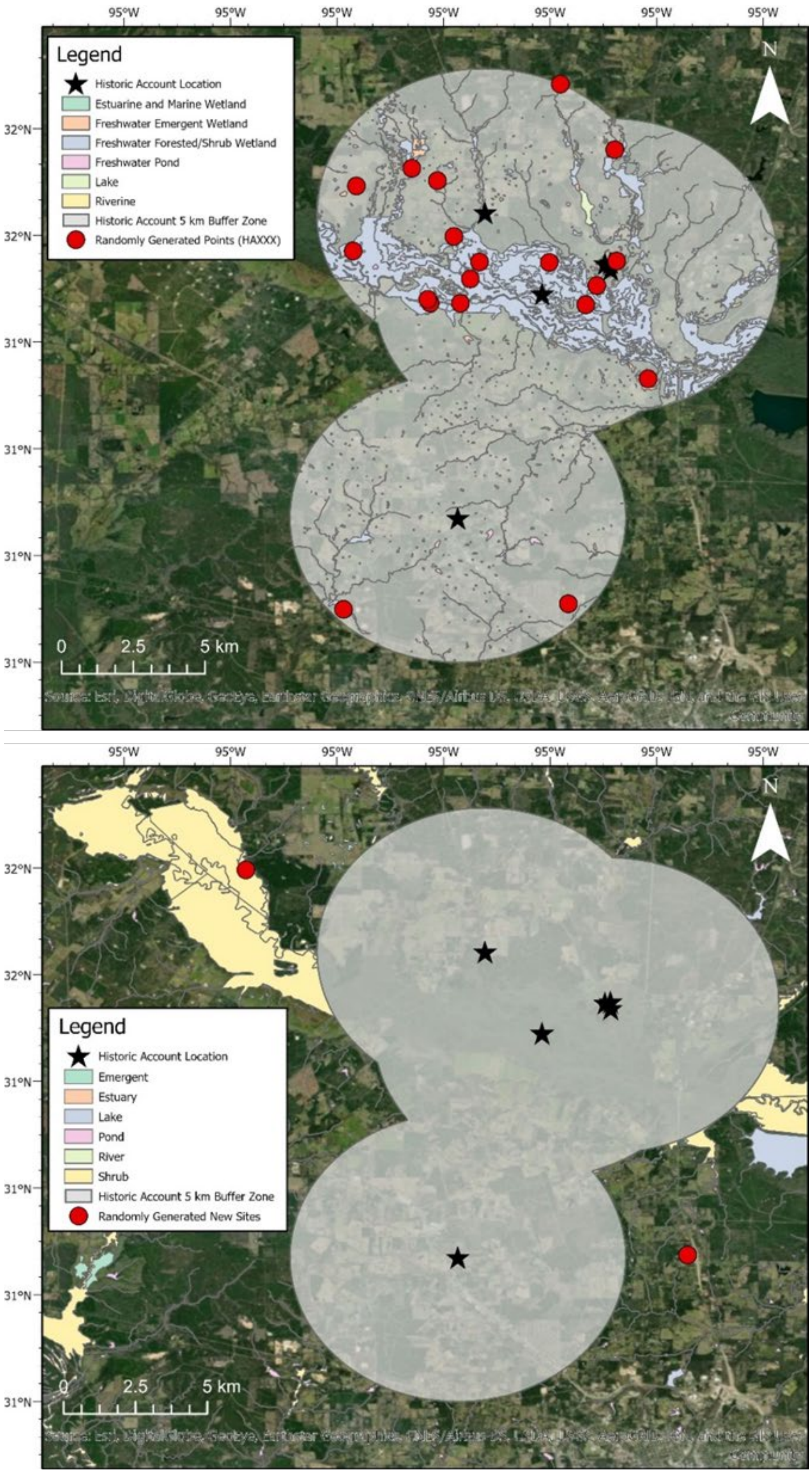


Figure 2 Example of randomized coordinate generation for Historic Account (top) and Historic County (bottom) field survey locations.

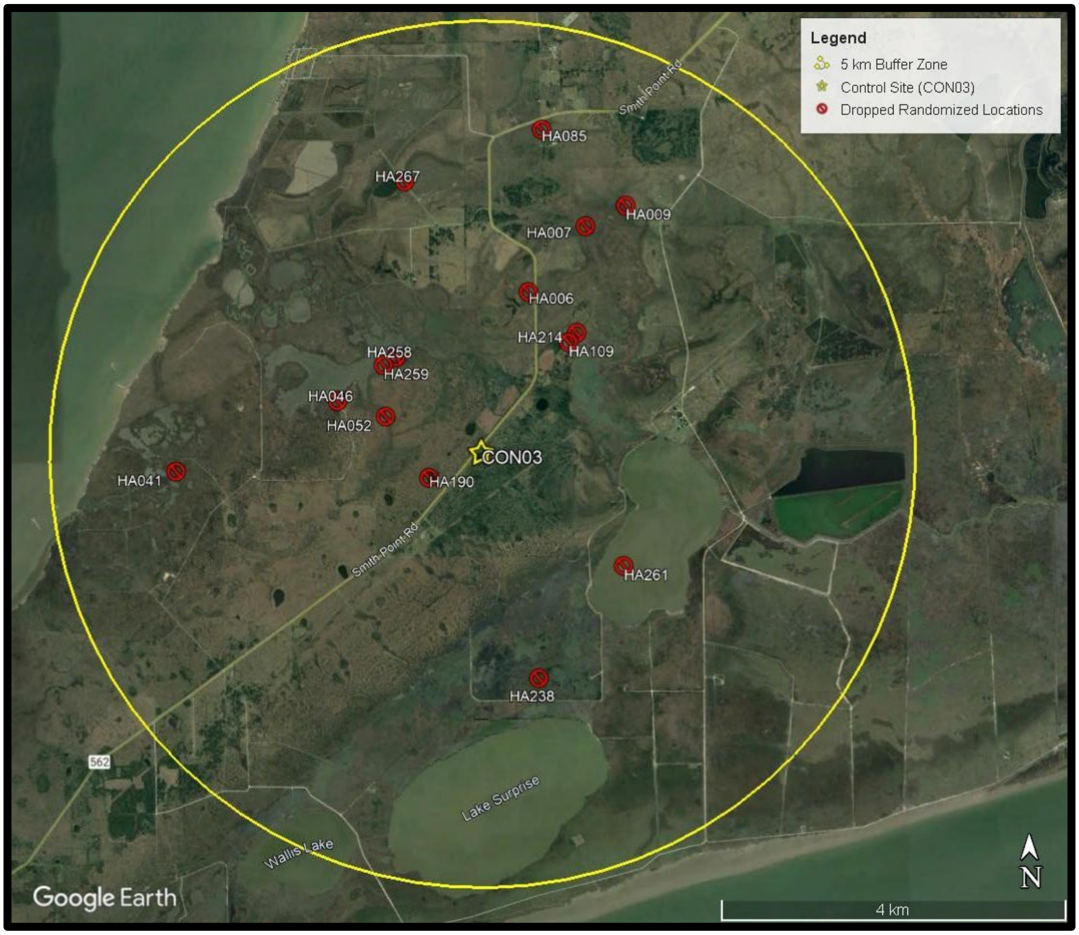
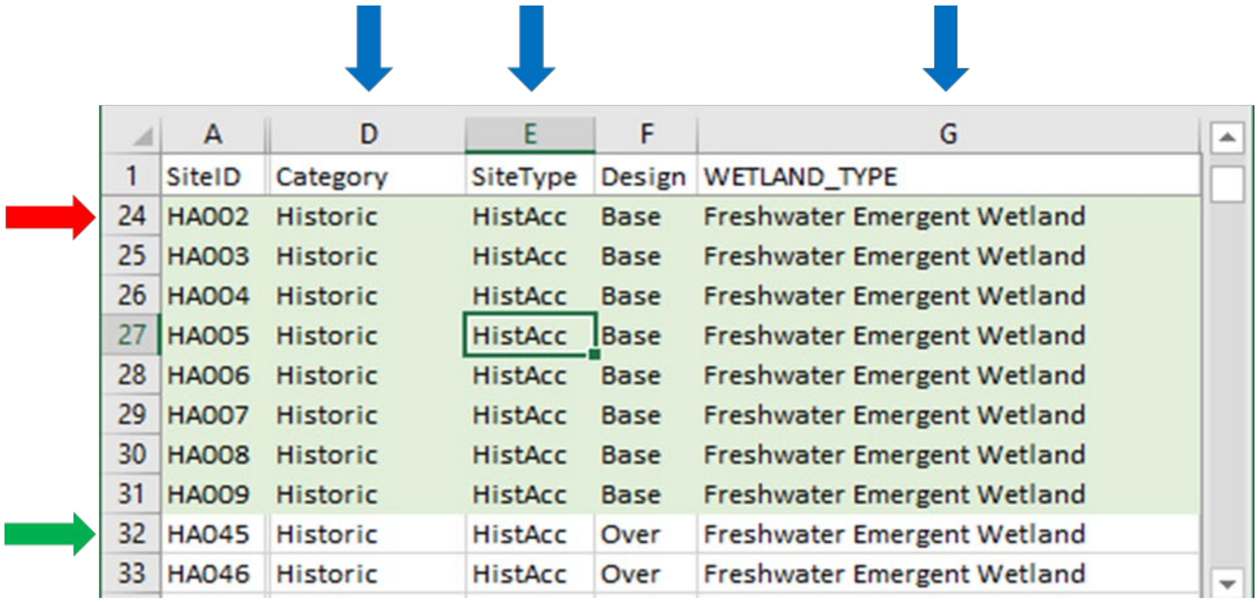


Figure 3 Example of randomized locations dropped from within a 5-km buffer (yellow circle) of a Control site yellow star). Dropped locations indicated by red circles with cross-through.

Table 3 Description and examples of reason(s) randomized survey locations would be dropped from the sample design. Classifications from National Wetland Inventory (NWI; USFWS 2019).

Reason for Drop	Description	Example
Within 5-km of Control	Randomized location(s) within a 5-km buffer of Control site dropped to increase spatial distribution of randomized locations.	See Figure 3 for example of Historic Account location distribution within a 5-km buffer of a control site location.
Non-target	Randomized location(s) generated in areas no longer meeting classification based on aerial imagery within 500 m of randomized coordinates (location dropped if no comparable habitat available).	Site generated within a recently constructed parking lot; if a target wetland was identified within 500 m of the site, the sample location was shifted to that wetland.
Access Denied	Landowner(s) denied access to property verbally, digitally, or via distributed paper permission form.	Access was denied verbally; an electronic response denying access was returned; a paper permission form was returned with “access denied” checked on the form.
Unable to Contact Landowner	Specific landowner contact information unattainable; no response from landowner after multiple (≥ 3) attempts.	No response to emails, voicemail messages, or mailed letters.
Inaccessible	Randomized location(s) generated in areas inaccessible due to geographic barriers.	Site generated middle of shallow, non-boatable or walkable swampy area; if a target wetland was identified within 500 m of the site, the sample location was shifted to that wetland



	A	D	E	F	G
1	SiteID	Category	SiteType	Design	WETLAND_TYPE
24	HA002	Historic	HistAcc	Base	Freshwater Emergent Wetland
25	HA003	Historic	HistAcc	Base	Freshwater Emergent Wetland
26	HA004	Historic	HistAcc	Base	Freshwater Emergent Wetland
27	HA005	Historic	HistAcc	Base	Freshwater Emergent Wetland
28	HA006	Historic	HistAcc	Base	Freshwater Emergent Wetland
29	HA007	Historic	HistAcc	Base	Freshwater Emergent Wetland
30	HA008	Historic	HistAcc	Base	Freshwater Emergent Wetland
31	HA009	Historic	HistAcc	Base	Freshwater Emergent Wetland
32	HA045	Historic	HistAcc	Over	Freshwater Emergent Wetland
33	HA046	Historic	HistAcc	Over	Freshwater Emergent Wetland

Figure 4 Example of Base and Oversample site replacement for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) surveys in east Texas. Red arrow indicates dropped Base site (Site ID = HA002); Green arrow indicates replacement site (Site ID = HA045); blue arrows point to columns containing matching replacement criteria (site category, site type, and original NWI-derived wetland classification).

Non-Randomized Survey Site Selection

In addition to randomized site locations, non-randomized site locations were divided into three categories (Table 1): locations with ongoing WCT assessments or areas of known occupancy (“Control”), locations added opportunistically as new observations or potential habitats were identified throughout the project period (“Opportunistic”), and sites sampled as part of a supplemental study funded by the Sabine River Authority (“Supplemental”). Sampling at control sites (sites labeled as “CON”) was coordinated with researchers conducting ongoing surveys of WCT at select locations across the species range. Sites categorized as Opportunistic were included throughout the survey period as they were discovered either by personal communications, detections resulting from reports provided to the Online Reporting Tool (discussed later in this section), or reports made to online resources after the beginning of the project (e.g., iNaturalist reports, social media reports, etc.). Supplemental sites were selected with the goal of expanding visual, eDNA, and drone surveys to areas associated with major waterbodies within the Sabine River Basin. Sample locations at supplemental sites were selected based on presence of target wetland habitat (Table 2) and accessibility for establishing safe launch and land points, allow for line-of-sight with the drone to be maintained, and have sufficient wetted area to survey.

In- and Out-of-Season Sampling Periods

It has been hypothesized that the most-likely window of activity for the WCT within its range in Texas is between March-July (Ryberg et al. 2017, Bowers et al., 2021, 2022a; P. Crump, Texas Parks and Wildlife Department, *personal communication*). Therefore, for the purpose of this study, we divided sampling periods between in-season (March-July) and out-of-season (August-February). During the in-season sampling period, all survey protocols were attempted and the in-season period was sampled across all three years (2020-2022). Out-of-season sampling was conducted between August 2021-February 2022 to determine if WCT were still active outside of the presumed activity period. During the out-of-season period, only BAVS and eDNA were conducted at four sites: CON02a, CON02b, CON03, and OPP02. These sites were selected due to confirmation of WCT occupancy across multiple sampling events in the previous two years of in-season sampling and their relative distance from the University of Houston-Clear Lake (UHCL) campus.

Detection Protocols

A combination of field survey protocols were used to assess habitat associations and test efficacy and efficiency for detection of WCT. Due to logistical difficulties in implementation of some protocols (e.g., walking surveys, drone surveys, hoop trap- surveys, and canid scent surveys), not all protocols were implemented at all sites. Protocols for small-scale habitat data collection, binocular assisted visual surveys, and environmental DNA sample collection were conducted at every site during every site visit. The following describes specific methodologies for these sampling protocols.

Small-scale Habitat Data Collection

General site characteristics were recorded during each sampling event. Site coordinates (decimal degrees, datum WGS84) were recorded at the water's edge (or perceived water's edge when water was not present) using a handheld GPS unit (Garmin eTrex 10, Garmin Ltd., Olathe, Kansas). Environmental, habitat and water quality data were collected in reference to this point, herein referred to as the "assessment point" (Figure 5A), and were documented following protocols outlined in the Texas Commission on Environmental Quality (TCEQ) Surface Water Quality Monitoring (SWQM) Manuals, unless otherwise noted (TCEQ 2012, 2014). Data were recorded from within a 10 m x 10 m plot centered around the assessment point, with a portion of the plot extending over the wetted area, and a portion of the plot extending over the shoreline/terrestrial habitat (Figure 5B).

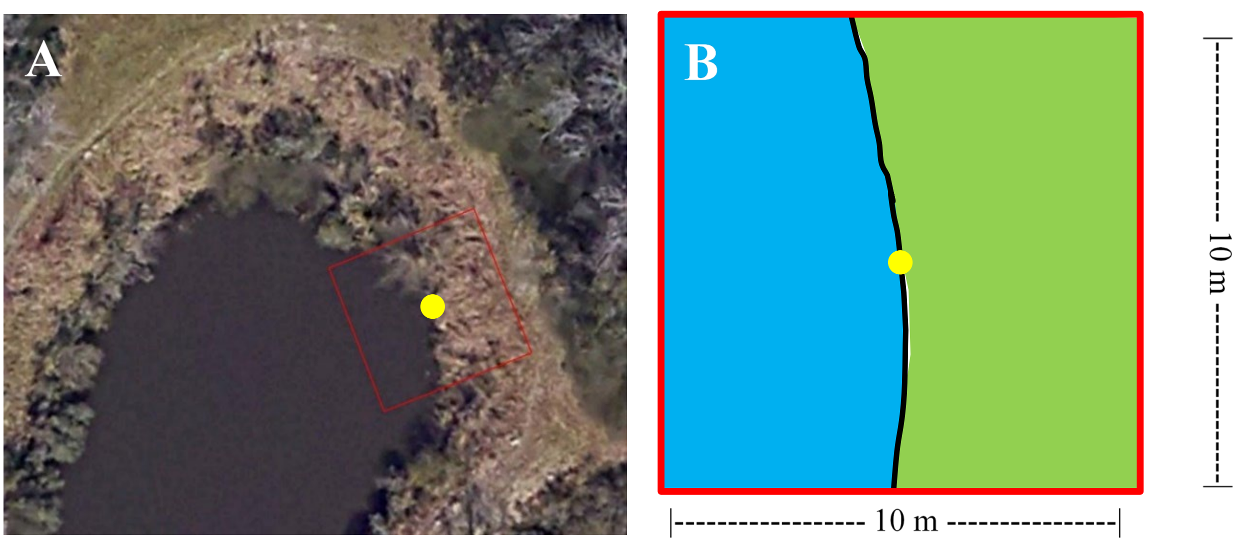


Figure 5 Example of 10 x 10 m site and habitat assessment plot. Panel A: GoogleEarth aerial imagery with plot (red square) around assessment point (yellow circle). Panel B example of site assessment plot with approximately 40% water coverage (blue) and 60% vegetation (green).

Environmental conditions included current weather, estimated 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 (e.g., in the time period prior to field sampling). Riparian canopy cover was visually divided into three layers (upper-canopy = > 5 m vegetation height, mid-canopy = 0.5-5 m vegetation height, and lower-canopy = < 0.5 m vegetation height) (Figure 6). Dominant vegetation type, percent cover of dominant vegetation, and percent cover of all vegetation were recorded as two-dimensional aerial coverage for each layer (Figure 7). Overall percent canopy cover was calculated using a convex spherical densiometer (Mills and Stevenson, 1999) (Figure 8).

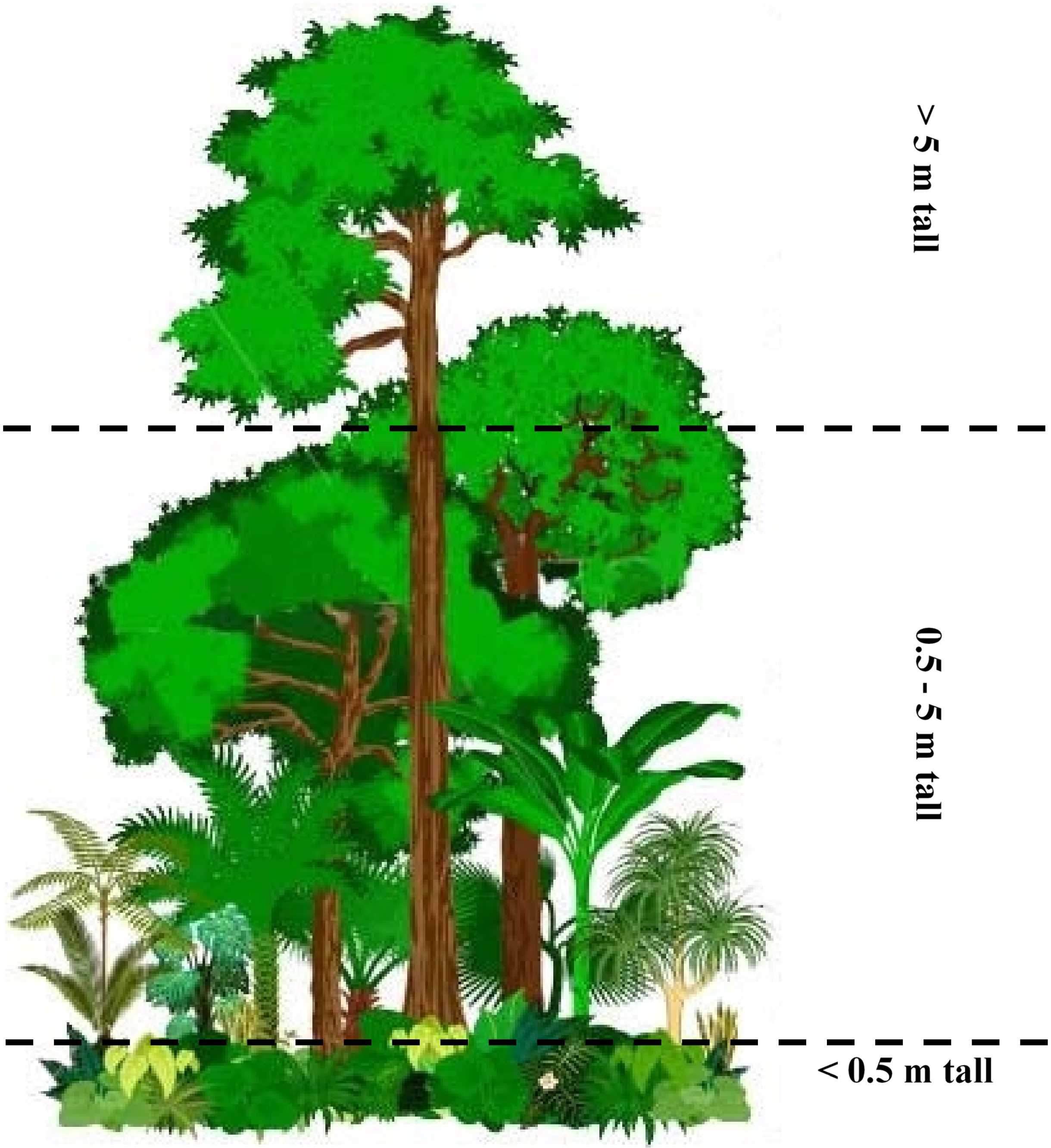


Figure 6 Division of canopy layers for upper- (> 5 m), mid- (0.5-5 m), and lower- (< 0.5 m) canopy cover estimates.

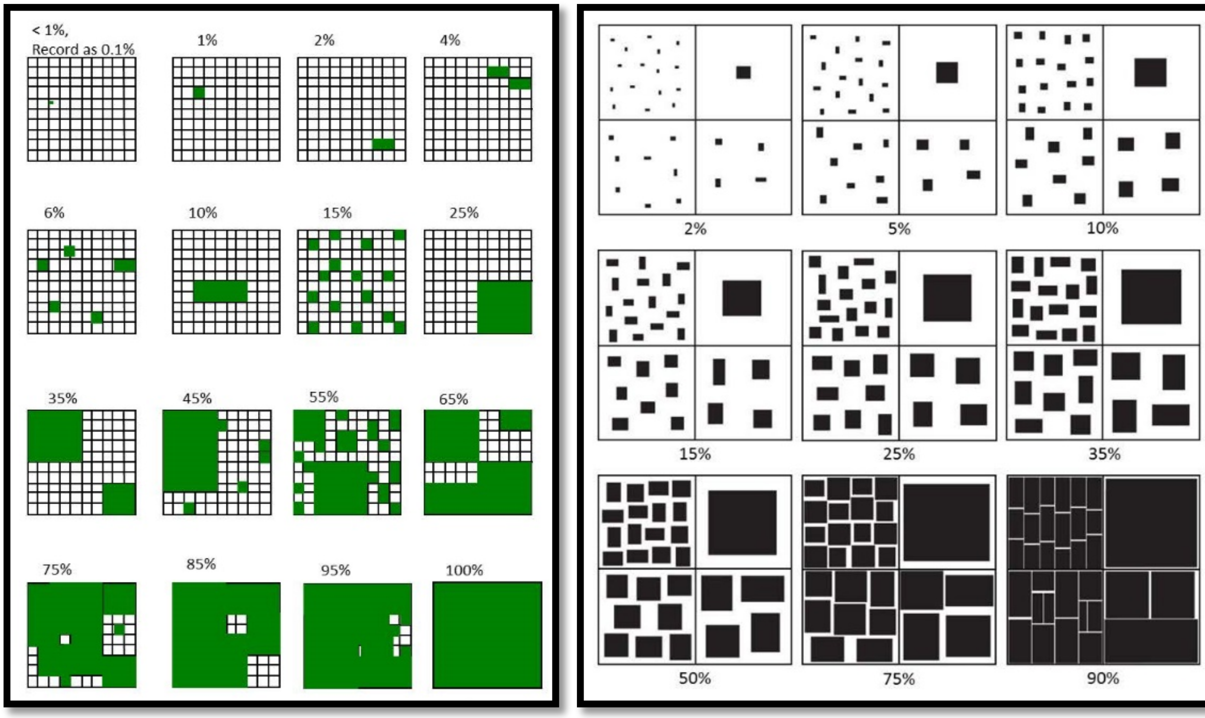


Figure 7 Examples of guides used for percent cover estimates.

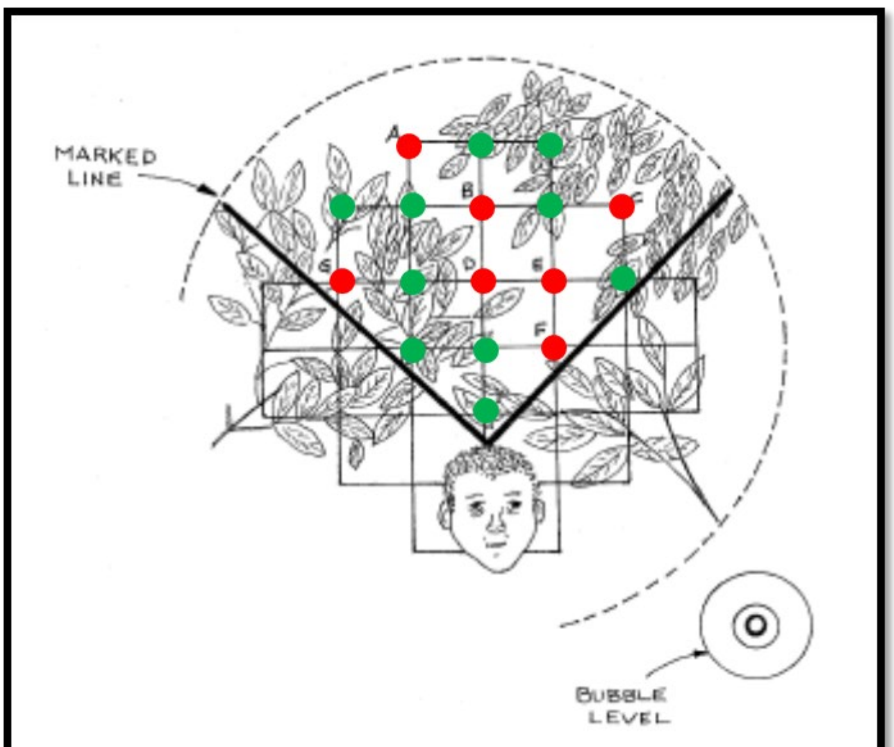


Figure 8 Example of canopy cover estimate calculated using a spherical crown convex densiometer (Mills and Stevenson, 1999). Green dots represent intersections of gridlines with canopy vegetation (n_v = 10). Red dots represent gridline intersections without canopy cover touching them (n_e = 7). Total percent cover in this example = $[n_v / (n_v + n_e)]$ = 10 / 17 = 58.8%.

Water quality variables were recorded (when water was present) adjacent to the assessment point using a multiparameter sonde (ProDSS, YSI and Xylem Inc., Yellow Springs, Ohio) suspended at half the total depth (m) and included: collection time, total depth (m), 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 recorded using a 1.2-m Secchi tube. A 125 mL surface water grab sample was collected for turbidity analysis (NTU) with a portable turbidimeter (2020we Turbidimeter, LaMotte Co., Chestertown, Maryland). Holding time for turbidity samples did not exceed 15 minutes.

Binocular Assisted Visual Surveys (BAVS)

At all sites, prior to establishment of the assessment point, binocular assisted visual surveys (BAVS) were performed for a minimum of 20 minutes in an attempt to pre-emptively confirm presence of WCT (Figure 9). Field personnel established a stationary location (or multiple locations if visibility was limited) along the boundary of a waterbody and conducted surveys by scanning a 180° plane (facing the water) using binoculars or a spotting scope. Areas providing opportunities for basking were prioritized, though open water was also monitored for observations of swimming and breaching individuals. Survey duration at each location was recorded (minutes) and observation(s) of aquatic herpetofauna were recorded. For each observation, time, distance from the survey point (m, taken with a range finder), bearing from the survey point (degrees, recorded with a magnetic compass), species (to lowest taxonomic level observed), confidence of species identification (Table 4), number of individuals observed, and behavior or activity were recorded.

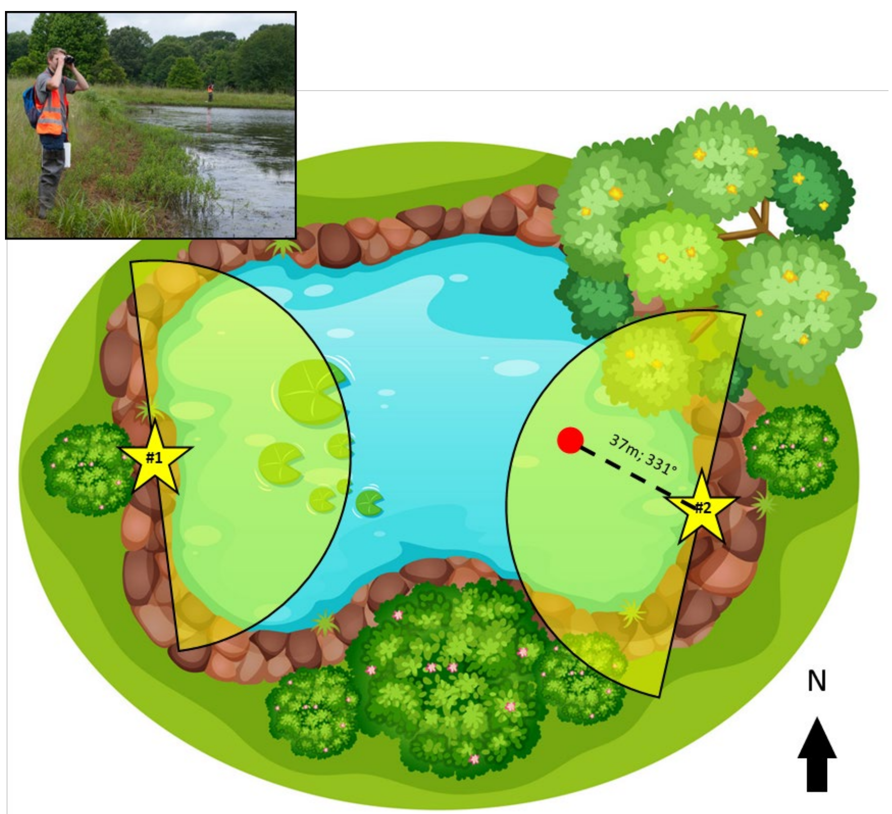


Figure 9 Example of binocular assisted visual survey (BAVS). Yellow stars indicate surveyor locations. Scanning area (180° plane) indicated by yellow half-circles. Herpetofaunal observation indicated by red dot. Distance and bearing calculation indicated by black-hashed line.

Table 4 Confidence levels ("Conf. level") used by observers during binocular-assisted visual surveys (BAVS). Low value (minimum = 0) indicates lowest confidence in identification and documentation of observation, high value (maximum = 3) indicates highest confidence in identification and documentation of observation.

Conf. level	Description
0	Observer able to see movement but unable to specify exact location of observation and/or reliably identify type of organism(s) to any taxonomic level.
1	Observer able to document general location of observation and/or identify major type of organism(s) (e.g., mammal, reptile, amphibian, etc.) but unable to reliably identify to lower taxonomic group.
2	Observer able to accurately document location of observation and/or identify organism(s) to lowest taxonomic level.
3	Observer able to accurately document location of observation and most-confident in identifying organism(s) to recorded taxonomic level.

Environmental DNA (eDNA) Surveys

Field sample collection

Environmental DNA (eDNA) surveys targeted at detecting WCT were conducted during all site visits. Up to three matrix samples were collected depending on site conditions. At sites where water ≥ 10 cm was present, two water-matrix grab samples were collected: 1) undisturbed, ambient water within the upper half of the water column and 2) re-suspended sediment (top 1 cm of sediment resuspended in water column; sample taken from resulting plume). Water samples were collected at four equidistant (10 m) locations in 20–40 cm water (when possible) along the waterline and composited prior to filtering (Figure 10). At Control sites and sites where no water was present, soil-matrix samples were also collected. Soil-matrix samples were collected at three equidistant (10 m) intervals along the bank approximately one meter above the visible (or perceived, if no water was present) water line using a stainless-steel teaspoon decontaminated with 50% bleach solution. Each collection of the upper 1–2 cm of soil was directly deposited into a pre-labelled Whirl-Pak to avoid potential cross contamination. At non-control sites where WCT were observed, a soil-matrix sample was also collected at the observation location. For each water-matrix sample, water depth (cm) and sample type were recorded. For soil-matrix samples, the final weight of the composite sample was recorded.

Sample bottles were pre-labelled and packaged individually for each site in order to minimize potential for contamination while in the field (Figure 11). Additionally, samples were stored on ice in separate coolers for each site for transportation to the lab prior to filtering to minimize the possibility of cross contamination via ice-melt water. Between sample sites, gear (including waders and booties) were decontaminated using a 10% bleach solution and allowed to dry to avoid genetic cross contamination between locations. All sample bottles and coolers were soaked in 50% bleach solution and allowed to completely dry before reuse in the field during later sampling events. No sample bottles were used for more than one in-season sampling period to avoid potential for cross contamination between years.

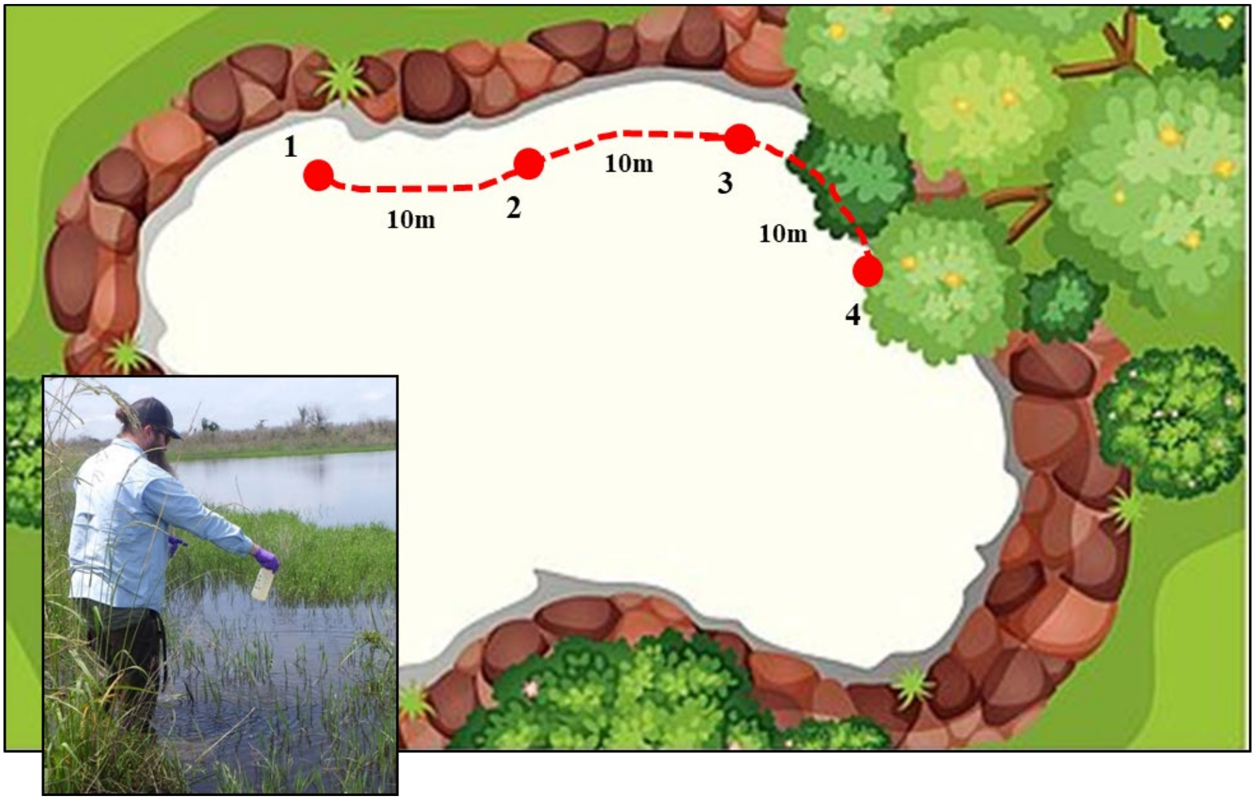


Figure 10 Example of water sample collection for environmental DNA (eDNA) analysis. Red dots indicate sample locations; hashed lines indicate 10 m distances between sample locations (following water's edge).

Figure 11 Example of pre-prepared sampling kits used for environmental DNA (eDNA) sampling (left) and self-contained cooler for each sample site (right). All site kits were pre-prepared in a decontaminated, designated space prior to sampling in order to mitigate potential for cross-contamination between sites.

Sample processing and laboratory analyses

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, a filter blank was collected using de-ionized (DI) water and a sterile, pre-loaded 0.45 µm cellulose nitrate (CN) filter. Water-matrix samples from control sites were filtered using two pore sizes: 0.45 µm and 3.0 µm. All other sites were filtered using a 3.0 µm CN filter. Sterile, pre-loaded 0.45 µm filter cups were used once and discarded. Glass filter apparatus' (Figure 12) and equipment reused between samples (forceps, homogenization containers, etc.) were soaked in 50% bleach, rinsed, and allowed to dry after each use. Filters were placed in individual Whirl-Paks pre-loaded with desiccant beads. Water filters and soil-matrix samples were stored at 4°C until they were shipped to Tangled Bank Conservation (TBC; Asheville, NC) for polymerase chain reaction (PCR) analysis. Soil-matrix samples were processed by TBC within 30 days of collection.

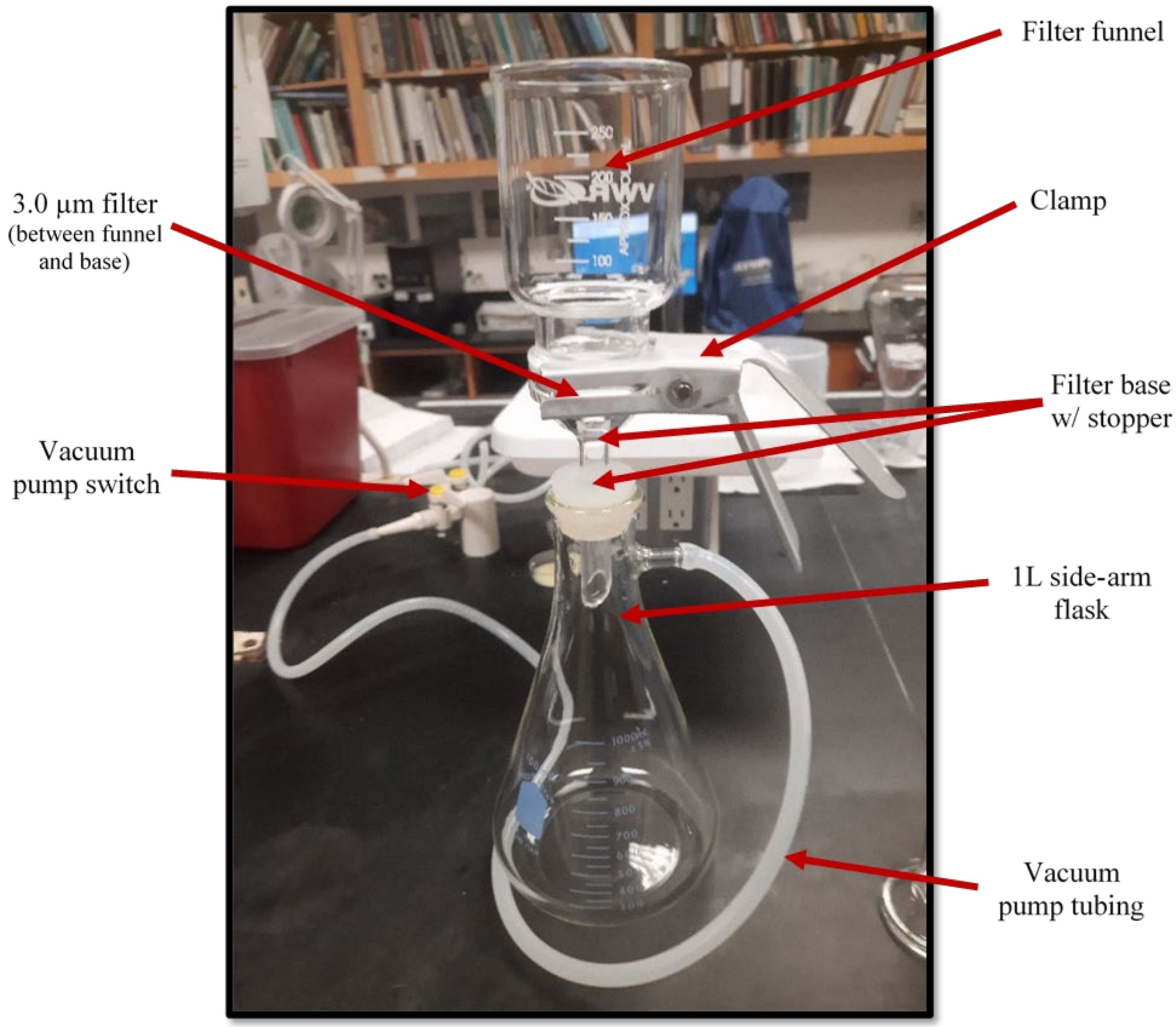


Figure 12 Example of glass filter apparatus used for processing environmental DNA (eDNA) water samples. Samples were processed in a dedicated space and all equipment were decontaminated with a 50% bleach solution and allowed to completely dry between filterings.

Filters were divided in half, with one half stored at -80˚C for potential later use. DNA was extracted from filters following methods described in Spear et al. (2015) with slight modifications of the DNeasy Blood and Tissue Kit (Qiagen) protocol. The standard extraction kit protocol was followed with addition of a Qiashredder (Qiagen) spin column after the lysis step. All samples were processed in a dedicated extraction and PCR section of the laboratory. Samples were amplified following methods described by Siler et al. (2020). A 74bp region was amplified using the following primers: 1) D_reticularia_CytB_F1 (CCTACCATGAGGCCA AATATCC);

2) D_reticularia_CytB_R1 (ATATATGGAATGGCT GAGAGGAGATT); and 3) probe D_reticularia_CytB_probe (AGGCGCAACTGTTA).

Most eDNA assays were performed via qPCR (Quant Studio 3, ThermoFisher). The eDNA extracts were run in a 20µL reaction volume consisting of 10µL of PerfeCTa qPCR ToughMix L-ROX (Quantabio, MA), 1µL of each primer at 10µM and probe at 5µM, 3.5µL nuclease free water, and 3.5µL of eDNA sample extract, or 1uL WCT positive control tissue extract with 2.5uL of molecular grade water. The qPCR thermocycler protocol is as follows: 15 min at 95˚C, 50 cycles of 94˚C for 60 sec and annealing at 60˚C for 60 sec with data collection during the annealing stage. Samples were first run with an internal positive control (TaqMan Exogenous Internal Positive Control Reagents, Thermofisher) according to the protocol. We treated any inhibited samples with OneStep PCR Inhibitor Removal Kits (Zymo). There was a slight protocol change in the middle of 2021. All samples processed after spring of 2021 were run with the description of Quant Studio 3, ThermoFisher listed above as opposed to the Applied Biosystems 7900HT system described below.

A 20 µL reaction volume (comprised of: 10 µL Luna universal probe qualitative polymerase chain reaction (qPCR) master mix, 1 µL of each primer (10 µM) and probe (5 µM), 3.5 µL nuclease free water, and 3.5 µL of sample extract) was run on an Applied Biosystems 7900HT system. The qPCR protocol was: 1) 15 minutes at 95˚C, 2) 50 cycles of 94˚C for 60 seconds, and 3) 60˚C for 60 seconds, with all data collected during the annealing stage at 60˚C. All extractions were run in triplicate and included a positive control from a WCT tissue extract and negative control to ensure PCR efficacy and identify potential contamination. Cycle threshold values were generated using SDS 2.4 software. 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.

Samples with a minimum of two replicate amplifications were deemed "positive" indicators of WCT presence, while single amplifications were noted as "potential" indicators of presence.

Walking Surveys (WS)

Walking surveys (WS) were conducted at sites where three or more field team members were present so they could be simultaneously performed with BAVS for comparability in data analyses. At sites that had obstructions affecting BAVS visibility, surveys were conducted by walking transects along the perimeter of the wetland (if water was too deep to walk through) or through the wetland. Start and end coordinates (decimal degrees, datum WGS84) and times were recorded for each survey. Similar to BAVS, WS were conducted for a minimum of 20 minutes. If a herpetofaunal specimen was encountered, coordinates (decimal degrees, datum WGS84), species (to lowest taxonomic level), count, behavior, and activity was recorded for each specimen. When possible, individuals were photographed prior to release.

Drone Surveys

Drone surveys were conducted at select sites using two platforms (Figure 13): a DJI Phantom 4 Multispectral ($Drone_{P4}$, multi-spectrum static imagery within the visible light spectrum, Figure 14) and DJI Mavic 2 Enterprise Dual ($Drone_{M2}$; visible- spectrum video imagery). Flights were performed by a Part 107 certified remote Pilot in Command (PIC) (Pilot #4465149) following Federal Aviation Administration (FAA) regulations and conducted under (TPWD Aerial Wildlife Monitoring Permit M2885) with proper landowner permission.

Figure 13 Drone platforms used for aerial surveys: DJI Mavic 2 Dual (Drone$_{M2}$; left) and DJI Phantom 4 Multispectral (Drone$_{P4}$; right).

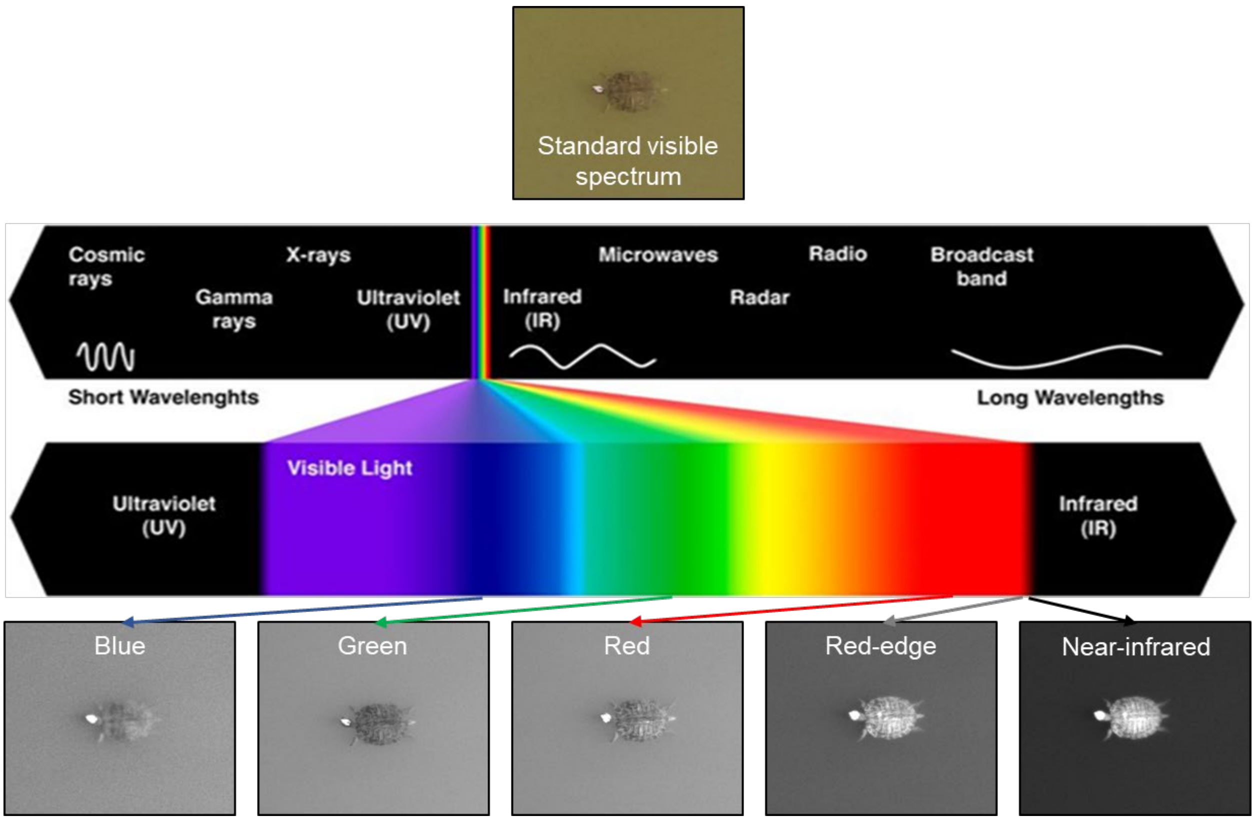


Figure 14 Example multispectral static imagery of a red-eared slider (*Trachemys scripta elegans*) taken with the DJI Phantom 4 Multispectral platform (Drone$_{P4}$) as it appears in the visible-light spectrum. Visibility of diagnostic characteristics increases at longer wavelengths as more energy is captured by the sensors. While the characteristic "red-ear" is not visible, shell shape and carapace markings can be used to confirm species, especially along green, red, red-edge, and near-infrared bands.

Prior to conducting aerial surveys, planned plot-transect flight paths were generated via the DJI Pilot application (iOS v1.1.5) and Litchi (iOS v4.25.0-g) for the Drone$_{M2}$ while DJI GS Pro (iOS v2.0.17) was used for the Drone$_{P4}$. Flights were conducted during daylight hours and we attempted to conduct aerial surveys at times when the sun was not at an extreme angle in order to avoid impacts of glare. 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 damage to the platform. Automated flight paths (e.g., not controlled by the pilot but visually monitored) were determined based on current environmental conditions and in a manner that allowed the pilot to maintain line of sight of the platform at all times. Manual flights (e.g., controlled by the pilot) 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 during manual flights. Flights were performed at a speed of 1 m/s with a target altitude of 5 m and -90° gimble (e.g., camera angled straight down). Slight gimble tilt (approximately -45°) was tested in situations involving skittish turtles, heavy glare or reflections, and in instances where overhanging vegetation obstructed the field of view. Static imagery with the Drone$_{P4}$ was collected with a 10% overlay (e.g., 10% of the frame overlapped between images) at an equal time interval of 2.26 seconds, so as to generate a full image of the survey area for data processing. 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).

At all sites, BAVS were performed concurrently with Drone$_{M2}$ surveys, with Drone$_{P4}$ surveys conducted immediately following Drone$_{M2}$ surveys. During drone surveys, behavioral response to the platform and estimated linear distance from the unit were recorded when the individual(s) was flown over.

Static (Drone$_{P4}$) and video (Drone$_{M2}$) imagery 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 playback speed, and extract snippets or clips of video imagery for more conclusive analyses. 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. During video analyses, if an animal reacted to the Drone$_{M2}$ platform, the level of reaction was scored between 0-4 with 0 being least reactive and 4 being most reactive (Table 5). If a reaction was indeterminable from video analysis, a score of "Unk" (e.g., unknown) was recorded.

Table 5 Reaction score, description, and example(s) of behaviors observed during Drone$_{M2}$ video analyses.

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 before/after drone platform passed or during platform elevation decrease/increase
3	Submergence and retreat	Submerged and swam away to cover or out of view of the drone imagery
4	Quickly retreated	Submerged rapidly creating a splash or obvious water disturbance during submergence
Unk	Unknown	Unknown reaction, submerged before entering frame, ripples in edges of frame with no observable behavior in imagery

Trapping Surveys

Hoop trapping surveys were conducted at select sites using 20-inch (50.8 cm) diameter, 6-foot (1.83 m) long fiberglass hoop traps with a 1-inch (25.4 mm) square mesh. Three trap arrays were used per trapping event with each trap array consisting of two hoop traps anchored at each end of a 14-foot (4.3 m) long, 3.8-foot (1.2 m) height seine with 0.04-inch (1 mm) mesh (similar to Bowers et al., 2022a). Cumulative trapping effort (number of trap nights multiplied by number of traps deployed) and total number of captures were used to calculate catch per unit effort (CPUE; number of individuals per trap night). Traps were deployed without bait at the recommendation of other WCT researchers in Texas (B. Bowers, Texas A&M University, College Station, *personal communication*).

Game Camera Trap Surveys

Game cameras "traps" were installed at select sites using a floating wooden platform fixed in front of a Reconyx HyperFire 2 Covert IR game camera (model: HF2X Gen3; Reconyx, Holmen, WI, USA) mounted to a U-post staked in the substrate. Cameras were set to the highest sensitivity with three-image bursts per trigger event. In addition to motion triggers, cameras were set to automatically capture imagery once every hour in order to assure that the camera was still operational during long periods without motion triggers. Cameras were equipped with lithium-ion AA batteries (Ultimate Lithium, Energizer Brands LLC, St. Louis, MI, USA) and a 32-GB SD card to allow cameras to operate continuously. In Year 2, traps were deployed for 24-hours each month as an initial test of the method. In Year 3, traps were deployed continuously during the full in-season survey period. During continuous deployment, photos were downloaded and batteries replaced (if needed) once per month, during each site visit.

Canid Scent Surveys (CSS)

Canid scent surveys (CSS) were conducted at select sites using trained conservation detector dogs. Detection training follows a multi-step process during which the individual dog is assessed for ability to perform the protocol. At the onset of training, the dog is exposed to a turtle contained in a suet-type cage allowing for the turtle to be visible, unable to escape, and protected. Once the dog observes the turtle, a reward is provided (toy or food). The detection-reward cycle is repeated in order to imprint on the dog that "turtle = reward". Empty cages are added to a lineup to reinforce "empty cage ≠ reward". Further, to train the dog to present a "passive alert", the reward is withheld until the dog looks to the handler. At that moment, the dog is given a command to "sit" (or "down") and, once the dog offers the requested behavior, the reward is provided. As training progresses, visibility of the contained turtle is slowly decreased, which forces the dog to incorporate olfactory detection. Once the dog is able to reliably detect hidden, contained turtles, training progresses to the field.

For this study, detector dogs were initially trained using Eastern Box Turtles (*Terrapene carolina carolina*). Once training progressed to field work, the dog(s) were rewarded for any turtle encountered. This generalized approach increases the likelihood of getting a reward, especially when the target is uncommon or rare, and helps to maintain a high willingness to work; an important factor to avoid regression in training due to boredom or limited detections. Initially, a single golden retriever ("Raine") was used for surveys (Figure 15). This particular dog has a high detection rate using air scent, tracking, and visual cues. In Year 3 (2022), a border terrier ("Ghillie") was added to survey efforts. This breed demonstrates strong instinctive search behavior for burrows and tunnels, thus strengthening detection probability.

Figure 15 Conservation detection dogs used for canid scent surveys.

Surveys were conducted as close to the same time as eDNA, BAVS, and other methods, when possible. In some cases, when weather conditions were declining or did not allow for CSS surveys to be conducted concurrently (e.g., heavy precipitation, increased heat and humidity in summer months, etc.), CSS were conducted on the day prior to or post- field sampling. Search area was tracked using a Pathfinder Mini GPS-enabled collar (Dogtra, Garden Grove, CA) linked to an application on the handler's mobile device. The detector dog was allowed to stop periodically for water breaks and to allow the animal to rest (typically every 30 minutes, though times varied based on current environmental conditions).

Environmental parameters were recorded at chest and ground (0.3 m from substrate) level throughout the survey duration. Environmental parameters included time, location (decimal degrees, datum WGS84), air temperature (°C), wind speed (mph), wind direction (cardinal direction), and relative humidity (%). Survey start and end locations (decimal degrees, datum WGS84), total survey duration (including on- and off- survey times), "on-survey" duration (e.g., time spent actively searching), number of detections, location(s) of alert behavior(s) (e.g., physical changes in detector dog's demeanor if a scent was detected) and type of detection ("turtle found" or "animal not found") were also recorded. If a turtle was found, time, location (decimal degrees, datum WGS84), species, sex (if possible), and size class (hatchling, juvenile, adult, or unknown) were documented. A macrohabitat type was determined based on the general habitat type of surrounding area (residential, agricultural, forested, etc.). At each capture location, a microhabitat assessment was conducted in a 1-meter square plot centered around the turtle. Distance from water (m), substrate type (clay, muck, sand, organic, other), and percent ground cover for water, bare ground, vegetation, and duff were recorded in the 1 m^2 plot. Finally, air temperature (°C), wind speed (mph), wind direction (cardinal direction), and relative humidity (%) were also recorded at chest and ground level at the time of capture.

Road Surveys (RS)

Road surveys (RS) were conducted as conditions allowed. Start and end times, coordinates (decimal degrees, datum WGS84), and odometer readings were recorded for each survey.

Distance traveled (DT) was calculated by subtracting start odometer reading from end odometer reading. Relative composition of survey area habitat (industrial, residential, agriculture/rural, forested, urban, park, other) was recorded after each survey was concluded. All surveys were conducted when road conditions were safe and weather provided good visibility (e.g., no fog, rain, etc.). When specimens were observed, time, location (decimal degrees, datum WGS84), species (to lowest taxonomic level possible), count, behavior, water body type, and water presence was recorded. Certain circumstances allowed for stopping safely along the side of the road to let passengers to scan waterbodies thoroughly with binoculars.

Individual Data Collection

For all WCT collected, living or dead, capture method, date, time, location (decimal degree, datum WGS84), and morphometric measurements (mm) were recorded. Additionally, individuals were photographed prior to release. Measurements included midline straight carapace length (mid-SCL), maximum shell depth (max-SD), maximum shell width (max-SW), midline plastron length (mid-PL), and plastron width (PW). Each individual was sexed (when possible), and weighed (kg). Females were palpated for presence of eggs and examined for eggs and follicles using ultrasonography using a Sonosite Vet-180Plus equipped with an C11 micro-convex linear transducer (Sonosite Inc., Bothell, WA, USA), if the opportunity was available. If captured alive, individuals were marked for future identification using two methods: (1) insertion of an internal passive integrated transponder (PIT) tag in the posterior left leg (Buhlmann and Tuberville 1998) and (2) external marking using a systematic pattern of drill holes along the marginal carapace scutes (Figure 16; adapted from Cagle, 1939; similar to P. Crump, Texas Parks and Wildlife Department, *personal communication*). For live individuals, a microhabitat assessment was performed in a 1-meter square plot centered around the capture location. Habitat data included vegetation type(s), estimated canopy cover within three height classes (similar to those described previously), air temperature (°C), and overall wetland type (matching the 6 previously described target wetland types). If the turtle was found in water, water temperature (°C), water depth (cm), width of the wetted area, and distance from the bank were recorded. Data recorded for dead individuals included location (decimal degrees, datum WGS84) and general notes about surrounding habitat.

In instances where WCT were observed but not physically collected, activity type (swimming, basking, walking), distance (m), bearing (°) to the turtle, and relative location of individual (land, log, water, etc.) were recorded. Search method, time of observation, and any notes regarding habitat, other species present in the same area, and reasons why the individual could not be captured were also documented. If possible, a visual assessment of the 1-meter by 1-meter square plot centered around the turtle's location was recorded as described above.

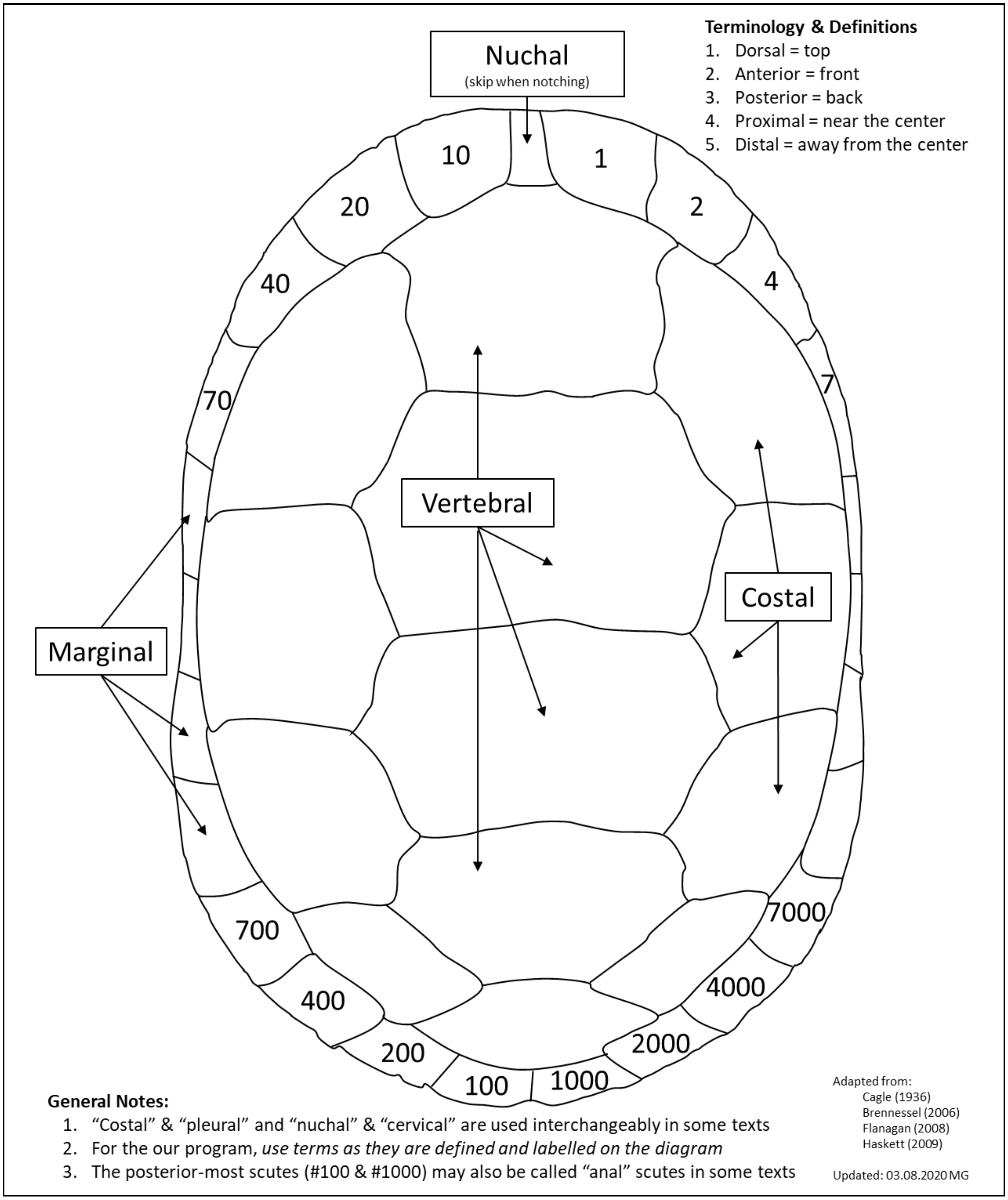


Figure 16 Pattern of external markings for Western Chicken Turtles (WCT).

Gear Decontamination Between Sites

An innate concern of state-wide assessments is transport of non-native or invasive species and pathogens between sampling locations. Decontamination protocols were based on those outlined in the U.S. Environmental Protection Agency's National Aquatic Resource Survey Field

Operation Manuals (example of protocols can be found in USEPA, 2018) and follow similarly to those outlined by the Declining Amphibian Task Force (DAPTF, 2021). Between sample locations and events, all vehicles, vessels, equipment, and field and personal gear were cleaned and allowed to dry (depending on the context in which gear was used and type of material from which the gear was composed). Cleaning solution(s) included high-pressure water, a 10% bleach solution, a phosphate-free cleaning solution, or 70% ethanol, depending on the material and application of the equipment used.

Online Reporting Tool (ORT)

A citizen science-based online reporting tool was developed in ArcGIS Survey123 using ArcGIS Online (ESRI 2021b) for use in Years 2 and 3 of the study. A full example of the ORT layout can be found in Appendix C. To summarize, reporters were required to fill in most sections of the ORT and could remain anonymous or provide contact information upon completion of the report. The first section of the ORT defined the goal of the tool and notified the reporter of where the tool was developed. The second section included a turtle identification guide and required reporters to confirm that their observation was of a wild WCT. The third section allowed reporters to provide a specific location of the observation (using a clickable and zoomable map), year and month observed, number of WCT observed, condition (alive or dead), microhabitat type (freshwater pond, river, lake, etc.), macrohabitat (via dominant land use type in surrounding area), behavior observed (swimming, basking, etc.), perceived general population trends (increasing or decreasing), and additional anecdotal information related to the observation. In each response of section three, field options would toggle based on the reporter's selection(s). Once a report was completed, reporters were given the option to make another report. Throughout each section, certain fields were required and the reporter could not submit their responses unless these fields were completed. Reporters were also allowed the opportunity and encouraged to provide photographs or files associated with the report. A progress track was provided so the reporter could track their completion status within the reporting tool.

Distribution of the reporting tool relied on "word of mouth" and social media. An email list was developed for distribution of the questionnaire to state wildlife biologists, regional conservation managers, refuge biologists, herpetological experts, wildlife rehabilitation staff, university research groups, state water authority personnel, landowners, wildlife society members, eco-tourism guides, master naturalists, zoo staff, veterinarians, etc. An electronic letter with the link and QR code for the online reporting tool was distributed multiple times over both years of the tools access. Additionally, fliers with the reporting tool information were handed out to interested individuals during the sampling season, social media posts about the reporting tool were posted to EIH's social media accounts, and information about the reporting tool was presented at meetings, conferences, and through various media outlets.

Report responses were reviewed for accuracy, completeness, and submitted photographs were checked for correct species identification. Reports not including photo-verified accounts of WCT were excluded from data analyses, including species distribution models (discussed later in this section). Additionally, locations where a WCT was accurately reported in Year 2 (e.g., included a photo of a WCT) were flagged for candidate Opportunistic field sites in Year 3.

Data Analyses

All data were compiled in Microsoft Excel 2016 for Windows and plotted using Excel, R, RStudio, and SigmaPlot v14.5. Specific R and RStudio packages are noted in the sub-sections below. Maps were generated using ArcGIS Pro (ESRI 2021a). Unless otherwise noted, statistical analyses were performed with α-values set to 0.05. All data were tested for normality and equal variance prior to analyses (Shapiro and Wilk, 1965). If data were determined to be non-normal or have unequal variance, non-parametric analyses were used. Due to small sample size, morphometric comparisons between male and female WCTs were conducted using One-Way Analysis of Variance (ANOVA) with Fisher LSD pairwise comparison or Kruskal-Wallis One-Way ANOVA on Ranks. In all instances, averages are presented as ± 1 standard error (SE) followed by range (minimum to maximum value) in parentheses. Boxplot boxes show inclusive 25^{th} and 75^{th} quartiles with whiskers representing the 1.5x interquartile range, points indicate outliers, and the line within each box represents the median. Letters above boxes represent significant groups (when detected).

Prior to data analyses, sites were assigned an overall occupancy status (across all years and sampling events). Sites were determined to be "Occupied" if one or more survey methods resulted in confirmed presence of WCT at any time during the study. Sites were determined to be "Potentially occupied" if eDNA samples resulted in only potential detections without confirmation of presence by another protocol. Sites with no potential or positive eDNA results or without confirmed presence using another protocol were assigned a final status of "No detections". For small-scale habitat and detectability analyses, independent sampling events were assigned binomial status values of 0 = no detections, 0.5 = potential detections, and 1 = confirmed detection. To better understand the relationship between small-scale habitat variables as predictors of occupancy and detectability by sampling event, sites with events assigned a status = 0.5 were removed prior to analyses.

Small-scale Habitat Analyses

Small-scale habitat statistical analyses were conducted using R 2022.07.2 (RStudio Team 2021). The relationship between events with status = 1 (WCT detected) versus events with status = 0 no WCT detected) were evaluated to determine the site characteristic(s) that maximized their detection and predicted occurrence using either Kruskal-Wallis rank sum test with subsequent post-hoc Pairwise Wilcoxon rank sum test (Bauer, 1972; Hollander and Wolfe, 1973) or binomial Generalized Linear Model (GLM) for detection prediction analysis (R package *pscl*). Multiple linear regression was conducted on environmental variables to determine which variables best explained the likelihood that an WCT would be detected at a site. Models were compared using Akaike Information Criterion (AIC).

Protocol Detectability Models

In order to determine best predictor models to calculate baseline detection probability (ρ) value for each protocol, we conducted single-season occupancy models in the R statistical package *unmarked* (Fiske and Chandler, 2011). A single-season occupancy model was used due to inconsistencies in the number of events and sites that a given protocol was performed during or at throughout the study period. All protocols were fit to the same series of co-variate tests to determine ρ for the best-fit model. Sampling event (as a factor of time) or effort (transformed around the mean) were used as observation-level co-variates. Due to inconsistencies in matrix design, these co-variates could not be assessed simultaneously. Site-level co-variates included surrounding habitat type, NWI wetland classification, and site selection criteria. Events resulting in no detections or potential detections, were assigned a binary value = 0. Confirmed positive detection(s) were assigned a binary value = 1. Models were run using detection data from sites with overall occupancy status "Occupied" in order to produce the best-case-scenario ρ-value for each method. A full list of the models used to test all protocols can be found in Table 6.

Table 6 Model co-variates used for standardized detectability models across all protocols. To standardized detectability models and calculation of detection probability (ρ), all protocols were tested using the applicable model co-variates in order to determine best predictor models based on Akaike Information Criteria (AIC) then tested for Goodness-of-Fit using Pearson's X^2 test.

Model #	ρ-type	Short Name	Model co-variates
fm0	none	fm0	Null
fm1	time	fm1.t	ρ (event)
	effort	fm1.e	ρ (effort)
fm2	none	fm2	Ψ (wetland)
fm3	none	fm3	Ψ (habitat)
fm4	none	fm4	Ψ (criteria)
fm5	none	fm5	Ψ (wetland+habitat)
fm6	none	fm6	Ψ (wetland+criteria)
fm7	none	fm7	Ψ (habitat+criteria)
fm8	none	fm8	Ψ (wetland+habitat+criteria)
fm9	time	fm9.t	ρ (event), Ψ (wetland)
	effort	fm9.e	ρ (effort), Ψ (wetland)
fm10	time	fm10.t	ρ (event), Ψ (habitat)
	effort	fm10.e	ρ (effort), Ψ (habitat)
fm11	time	fm11.t	ρ (event), Ψ (criteria)
	effort	fm11.e	ρ (effort), Ψ (criteria)
fm12	time	fm12.t	ρ (event), Ψ (wetland+habitat)
	effort	fm12.e	ρ (effort), Ψ (wetland+habitat)
fm13	time	fm13.t	ρ (event), Ψ (wetland+criteria)
	effort	fm13.e	ρ (effort), Ψ (wetland+criteria)
fm14	time	fm14.t	ρ (event), Ψ (habitat+criteria)
	effort	fm14.e	ρ (effort), Ψ (habitat+criteria)
fm15	time	fm15.t	ρ (event), Ψ (wetland+habitat+criteria)
	effort	fm15.e	ρ (effort), Ψ (wetland+habitat+criteria)

Number of sites (*N*), AIC, Akaike difference (ΔAIC), and Akaike weight (W_{AIC}) were calculated for each model. Goodness-of-Fit (GoF) tests were performed using Pearson's X^2 test on the top three models for each protocol. All GoF tests were run in 1,000 iterations and the *t*-score, mean, standard deviation (SD), and *P*-value were recorded for each test. For the model resulting in the highest *P*-value from the GoF test, the proportion of sites occupied (PAO), 95% confidence interval (95% CI), and detection probability (ρ) were calculated.

Protocol Comparison Rubric

In order to make recommendations towards efficiency and efficacy of each protocol employed during the current study, a comparison rubric was developed to equitably relate detection protocols resulting in different detection rates, effort, cost, etc. (similar to Riley et al. 2017) (Appendix D). Due to differences in logistical considerations to application of protocols, resolution of results or detections between protocols, and ranges of costs necessary to implement or conduct each protocol, criteria were divided into three broad categories: 1) Logistics (e.g., protocol development, permitting, and implementation), 2) Statistics (e.g., detectability, bias, and complexity of the protocol), and 3) Costs (e.g., money, time, and personnel). Each broad category was composed of multiple sub-categories which we considered influential in the implementation, detection, and analyses for a given protocol.

Logistical sub-categories

The Logistic category was divided into nine sub-categories (Table 7). For each sub-category, protocols were assigned a score of 0-4, with 0 being the least difficult and 4 being the most difficult. To reduce subjectivity and increase comparability between scores for a given sub-category, a matrix of scoring criteria was developed (Table 8). Even with detailed descriptions of scoring criteria, amongst individuals with more or less experience in a given protocol (or protocols), variation in the overall scores assigned to a given protocol or subcategory is likely.

Table 7 Sub-categories and considerations used in the Logistics category of the protocol comparison rubric

Sub-category	Considerations
Permissions	Effort to procure permits specific to protocol (including number of permits); effort to obtain access permission(s).
Planning	Level of intricacy for desktop planning, mapping, or software upload(s); need for field reconnaissance; level of intricacy of gear acquisition, preparation, and/or assembly.
Difficulty of gear transport	Difficulty in transporting gear due to mass, quantity, and/or overall size (dimensions) across the distance from access point to sampling area.
Difficulty of implementation	Movement distance needed while implementing protocol in sampling area (does not include considerations covered in "Difficulty of gear transport"); difficulty maneuvering through habitat while conducting protocol in given sampling area.
Time and maintenance	Time required to conduct protocol, includes maintenance of daily-use and installed remote sensing equipment.
Technical expertise	Technical knowledge and/or skills needed to implement protocol.
Performance variability	Degree to which protocol variability leads to missed or mistaken identification as a direct result of field personnel (e.g., “user-error”).
Potential for failure	Likelihood of apparatus malfunction, theft, and/or resultant missed or mistaken identification of individuals.
Resolution	Scale at which an individual can be identified (e.g., distinct individual, specific epithet, major taxonomic group, etc.).

Table 8 Standardized scoring criteria used for Logistical sub-categories of protocol comparison rubric.

Sub-category	Scoring Criteria (1 = "best"; 4 = "worst")			
	1 (none)	**2 (slight)**	**3 (moderate)**	**4 (extreme)**
Permissions	Does not require state or federal permitting, only access permit(s) or permissions necessary.	In addition to permit(s) from previous criteria, requires basic scientific permitting (state and/or federal); may require additional permitting for local or regional areas.	In addition to permit(s) from previous criteria, requires permit specific to protocol.	In addition to permit(s) from previous criteria, protocol requires specific training or certifications prior to being issued.
Planning	Protocol can be implemented upon arrival at sampling area with no prior knowledge of field conditions; gear prep is minimal.	Desktop evaluation or local knowledge is the only pre-sampling planning needed.	Application of protocol is contingent on environmental conditions (e.g., recent precipitation, low winds, etc.) and/or some pre-visit planning or gear prep is necessary.	In-person field reconnaissance required prior to implementation of protocol and/or protocol requires significant pre-planning and development.
Difficulty of gear transport	No scientific gear/equipment is needed and access to sampling area is easy.	Protocol requires limited gear/equipment (fits in small daypack).	Equipment requires multiple individuals to carry (due to size or weight) from access point to sampling area; large or heavy equipment may slow individual down while transporting.	Equipment transport requires multiple trips and individuals from access point to sampling area due to size or quantity of gear.
Difficulty of implementation	Minimal or no movement distance required (≤ 10s of meters); movement through habitat with limited obstructions.	Moderate movement distance required (≤ 100s of meters); movement speed slowed due to moderate deterrents.	Increased or variable movement distance required based on sampling area conditions; movement over varied terrain with some obstacles.	Long distances required (≥ 1 km); movement over steep or difficult terrain with numerous obstacles.
Time and maintenance	Protocol can be completed in ≤ 1 day with minimal equipment maintenance.	Initial set up and final tear down requires ≤ 2 days with ≤ 1 day of maintenance required per month.	Protocol requires time for initial set up or implementation > 2 days and > 1 day of continuous training or practice in order to maintain quality of protocol.	Protocol requires 3 or more days to implement and requires regular monitoring to avoid detrimental effects to wildlife.
Technical expertise	Protocol can be conducted by individuals with little/no experience prior to field work without direct supervision by expert.	Slightly challenging, minimal number of steps to complete, minimal concentration with some prior background knowledge or experience.	Complex, moderate number of steps to complete, requires some concentration and focus; requires previous experience and at least one field personnel trained specifically for gear type/protocol.	Extremely complex, many steps to complete, requires high level of concentration and focus, requires expert or highly trained personnel.
Performance variability	Little/no variability in detection or identification of individuals; all individuals able to be identified to specific epithet without chance for mistakes or contamination.	Protocol results in detections with range of confidence; most individuals can be identified to specific epithet, but some may only be identifiable to major group (class or family).	Most detections result in identification to major group (class or family), generally low confidence of identification to specific epithet.	Identification to specific epithet rare, identification to major group (class or family) can also be challenging.
Potential for failure	Little/no potential for failure, damage, theft, or misidentification by equipment; can be implemented at nearly any sampling area.	Low potential for failure, damage, theft, or misidentification by equipment but does happen with a measurable level of regularity; restricted by some rare site conditions.	Moderate potential for failure, damage, theft, or misidentification by equipment; highly variable and impacted by site common conditions that cannot be mitigated.	High likelihood of equipment failure, damage, theft, or misidentification by equipment; equipment/protocol is not able to be used at a considerable number of sites that otherwise would be included in study design.
Resolution	Individual can be identified to specific epithet and recognized as a distinct individual (e.g., data can be used for population estimates).	Individual can reliably be identified to specific epithet but not reliably identified as an individual (e.g., data cannot be used for population estimates).	Individual can be identified to genus or higher.	Individual can be identified to family or higher.

To standardize scores across a range of experience levels, Logistical sub-categories and the associated scoring criteria matrix were distributed to all project personnel who had participated in the current study. Respondents were asked to score the protocols for which they had familiarity with and use the considerations and criteria included for each sub-category. Scores were compiled and median score for each protocol and sub-category combination was determined. Any responses deviating more than ± 1.0 from the median score for each protocol and sub-category combination were removed (e.g., if the median value = 2.5, raw scores < 1.5 and > 3.5 were removed). A weighted average was then calculated for each remaining protocol and sub-category combination based on the number of responses, values provided for each protocol, and professional experience level of the respondent (weights: field technician = 0.25, crew leader = 0.5, project co-author = 0.75, project PI = 1.0). Protocols were then ranked from best to worst for each sub-category based on the weighted averages. Lowest averages were considered "best" (e.g., rank = 1) while highest averages were considered worst (e.g., rank = 14).

Statistical sub-categories

For each Statistical sub-category, values were calculated using the considerations and assumptions outlined in Table 9.

Table 9 Sub-categories, factors, considerations, assumptions, and ranking orders used in the Statistics category of the protocol comparison rubric.

Sub-category, factor, *(scale)*	**Calculation considerations**	**Calculation assumptions**	**Ranking order**
Number of personnel N_{pers} *(number, #)*	Cumulative number of personnel needed to conduct field work, sample processing, laboratory analyses, equipment maintenance and/or repair, and data compilation.	Minimum number of field personnel for all protocols = 2 (for safety reasons); minimum number of personnel for data compilation = 2 (two-step data entry and quality control process).	Low = best (e.g., 1); high = worst (e.g., 14)
Number of sites N_{sites} *(number, #)*	Number of sites that can be sampled per day.	Calculation only considers time in field (in hour); assumes 6-hour field day [8-hour total day with two hours for preparation, travel to/from field location(s), and decontamination protocol(s)].	High = best (e.g., 1); low = worst (e.g., 14)
Detection probability ρ *(proportion; 0-100%)*	Resulting ρ-value calculated from single-season detectability models using R package *unmarked*; final ranks based on best-fit model (X^2).	All models run using same ρ- and Ψ-dependent co-variates; models run using detection data for sites with confirmed occupancy only (0 = no detection or potential detection; 1 = positive detection)	High = best (e.g., 1); low = worst (e.g., 14)
"Catch" per unit effort CPUE *(number, #)*	Calculated as total number of positive detections divided by effort.	For protocols not resulting in effort as a function of time (e.g., eDNA or ORT), effort was calculated as number of samples or reports multiplied by values used for "Time (implementation)" from Cost category. For protocols resulting in effort as a function of time, effort was calculated in number of minutes.	High = best (e.g., 1); low = worst (e.g., 14)
Detection proportion Det% *(proportion; 0-100%)*	Proportion of positive detections made across all sampling events	Calculated as number of positive detections (potential detections excluded) divided by total number of events (or samples) protocol was applied to	High = best (e.g., 1); low = worst (e.g., 14)
Geographic coverage G_{cov} *(proportion; 0-100%)*	Estimated percent of available habitat sampled	Calculated as two-dimensional area covered by each protocol divided by the average[a] two-dimensional wetted survey area for sites where protocol was applied	High = best (e.g., 1); low = worst (e.g., 14)
Stages of analysis N_{stages} *(number, #)*	Number (count) of stages from data collection to detection confirmation	Stages [*n* = 10] assessed included: planning, permitting, preparation, fieldwork, sample processing, sample shipment, lab analyses, data compilation, data analyses, detection confirmation	Low = best (e.g., 1); high = worst (e.g., 14)

[a]For canid scent surveys, maximum wetted survey area was used in calculation as dog and handler were able to survey all habitats.

"Number of personnel" (N_{pers}) was calculated as the cumulative minimum number of personnel needed to complete each stage of a given protocol using the following equation:

Equation 1

$$N_{pers} = \sum (P_f + P_p + P_l + P_m + P_{dc})$$

where P_f is minimum number of personnel required for field work, P_p is minimum number of personnel required for sample processing (including sample shipment), P_l is minimum number of personnel required for laboratory analyses, P_m is minimum number of personnel required for equipment maintenance and/or repair, and P_{dc} is minimum number of personnel required for data compilation (including entry, check for quality control (QC), and preliminary data analyses, if needed).

"Number of sites" (N_{sites}) was calculated as the maximum number of sites that could be sampled in a given 6-hour field day. This calculation was based on estimates of implementation time (in hours) experienced in the current study and used the following equation:

Equation 2

$$N_{sites} = \sum \frac{T_{fd}}{T_{sh}}$$

where T_{sh} is total time (in hours) needed to implement a given protocol at the site and T_{fd} is total number of hours in a given field day. For our calculations, $T_{fd} = 6$ (see Table 9 for assumptions).

For "Detection probability" (ρ), probability models were compared using resulting AIC values. Predictability (ρ) values for the models resulting in the highest *P*-value from the Goodness of Fit test were used in the protocol comparison matrix. For protocols where ρ could not be calculated (e.g., ORT, WS, and RS), protocols were assigned ρ-values in relation to the number of WCT detected or reported using that particular protocol, but resulting in ranks lower than those of the protocols for which a ρ-value could be calculated. For example: WS did not result in any WCT detections and therefore a ρ-value could not be determined. Therefore, WS was given a ρ-value of "0.00". Conversely, RS and the ORT resulted in two observations and 10 reports, respectively, but a ρ-value could not be calculated due to the inability to assign a corresponding presence/absence matrix for both protocols. Therefore, RS and the ORT were given ρ-values of 0.01 and 0.02, respectively, which were lower than the lowest calculated ρ-value for all other protocols.

"'Catch' per unit effort" (CPUE) was calculated in two ways, depending on how "catch" and "effort" were determined. For protocols resulting in no physical captures or clearly defined time period, CPUE was determined using the following formula:

Equation 3

$$CPUE = \frac{Det^{+}}{\sum N_{samp}\ x\ T_f}$$

where Det^{+} is the total number of positive detections and effort is the sum of the total number of samples or reports (N_{samp}) multiplied by T_f which represents "Time (field)" from the cost-subcategory (calculation described below). For protocols resulting in physical captures and clearly defined time periods, CPUE was determined using the following formula:

Equation 4

$$CPUE = \frac{N_{cap}}{E}$$

where N_{cap} is the number of individuals captured and E is the total amount of effort in minutes. For example, E for hoop trap surveys is equal to the total soak time for trap arrays while E for BAVS is equal to the total amount of time spent surveying by all field personnel.

"Detection proportion" (Det%) was calculated as a proportion (0-100%) of the total number of samples or events conducted for a given protocol which resulted in positive (confirmed) WCT detection. In order to optimize protocol comparison, only occupied sites (occupancy confirmed by any method in the study) were considered for this calculation. The following equation was used to calculate Det%:

Equation 5

$$\sum \frac{Det^{+}}{N_{samp}}$$

where Det^{+} is the number of positive detections and N_{samp} is the number of samples or events from the "'Catch' per unit effort" sub-category.

"Geographic coverage" (G_{cov}) was calculated as a proportion (0-100%) of the two-dimensional spatial area sampled for each protocol. In general, G_{cov} was calculated as:

Equation 6

$$\sum \frac{A_{samp}}{A_{avg}}$$

where A_{samp} is the two-dimensional area (in m^2) sampled for a given protocol and A_{avg} is the average two-dimensional area (in m^2) of sites where the protocol was implemented. For eDNA samples, A_{samp} was calculated as:

Equation 7

$$eDNA^{water}: A_{samp} = (A_{bottle}\ x\ L) \quad or \quad eDNA^{soil}: A_{samp} = (A_{spoon}\ x\ L)$$

where A_{bottle} is the area of the bottle opening (area of a circle = πr^2; r = ½ diameter of bottle opening), A_{spoon} is the area of an ellipse (πab; a = length and b = width of spoon scoop), and L is the number of composited sample locations. For BAVS, A_{samp} was calculated as half the area of a circle using the following formula:

Equation 8

$$BAVS: A_{samp} = \frac{\pi (D_{CI3})^2}{2}$$

where D_{CI3} is the average distance (in meters) from surveyors reporting a confidence-level of 3 (see Table 4 for definitions of confidence intervals). During WS, only start- and end-coordinates were recorded for each survey. Therefore, distance traveled (in meters) was estimated as the maximum straight-line distance between start- and end-coordinates (represented by D_{max} in the formula below). To calculate sample area (in m^2) for WS, A_{samp} was calculated as:

Equation 9

$$WS: A_{samp} = (D_{max})(\pi r^2)$$

where πr^2 is the area of a circle centered around the surveyor with r equal to the visible area around the surveyor. For our calculations, r = 2 m. Road surveys were conducted opportunistically between sampling locations, at varying distances (spatial) and durations (time). For RS, A_{samp} was calculated as:

Equation 10

$$RS: A_{samp} = (D_{odom})(W)$$

where D_{odom} is the average distance traveled based on odometer readings and W is the estimated width of visibility on either side of the vehicle. For our calculations, we used 5 m to either side of the vehicle (10 m total, assuming surveyors on both sides of the vehicles). Because CSS are conducted by a team (including the detector dog and the handler) with members traveling at different speeds an over variable spatial areas, a weighted average was applied to determine the amount of two-dimensional area traveled by the detector dog (weight = 0.75) and handler (weight = 0.25); represented by D_{team} in the formula below. Additionally, because canid survey area was conducted in terrestrial and aquatic habitats, maximum two-dimensional surface area (A_{max}) was used to calculate geographic proportion (as opposed to A_{avg}). Furthermore, because the handler is responsible for monitoring the dog's actions and behaviors as well as monitoring spatial area within the immediate vicinity, area around the handler was scaled to a 1-m radius centered around the handler while area around the dog was scaled to a 2-m radius centered around the dog. This also accounted for increased detection ranges by the dog when using olfaction. Therefore, the proportion of geographic coverage for CSS was calculated as:

Equation 11

$$CSS: \sum \frac{A_{samp}}{A_{max}} = \frac{(D_{team})(A_{dog})(A_{hand})}{A_{max}}$$

where A_{dog} and A_{hand} are equal to the area of a circle (πr^2) centered around the specified team member. For our calculations r_{dog} = 2 m and r_{hand} = 1 m. Hoop traps can be set in any number of arrays, combinations, or even be moved during deployment in order to increase spatial extent. For this project, we used three trap arrays as described earlier in the methods. Spatial coverage of the traps was calculated as:

Equation 12

$$Hoop\ trap: A_{samp} = (L_{trap}\ x\ L_{net}\ x\ d_{mouth})$$

where L_{trap} is the total length of the trap, L_{net} is the length of the drift fence or fyke net between hoop trap openings, and d_{mouth} is the diameter of the mouth opening at the front of the trap. This calculation assumes that individuals may encounter the full length of the hoop trap, ultimately leading to the mouth and entry into the trap. It should be noted that not all species interact with hoop traps in this way, so this calculation should be updated to account for behavioral differences of other target species. Additionally, this calculation is specific to methods outlined for this study, which may not be applicable to future assessments utilizing hoop traps. Geographic coverage for Game Camera surveys was calculated as:

Equation 13

$$Game\ Camera: A_{samp} = (\frac{\pi(D_{view})^2}{6})$$

where D_{view} is equal to the radius of a circle matching the distance from the camera lens at which an individual can be reliably identified (we used 2.22 m). The Reconyx Hyperfire 2 has a visible angle of approximately 60°, so A_{samp} is equal to 1/6th the area of a circle.

For drone surveys ($Drone_{M2}$ and $Drone_{P4}$), a Ground Sampling Distance (GSD) calculator (www.pix4d.com) was used to calculate the survey area for one image frame (A_{GSD} = width of single image footprint on ground multiplied by height of single image footprint on ground). The $Drone_{M2}$ 2 unit collects spatial data in relation to duration of video footage (time). Conversely, the $Drone_{P4}$ unit collects spatial data in relation to duration and the number of frames recorded, applying a 10% overlap between frames during post-processing. Therefore, A_{samp} for each unit was calculated using the following equations:

Equation 14

$$M2: A_{samp} = \left(A_{GSD}{}^{M2}\ x\ T_{sec}{}^{M2}\right)$$

Equation 15

$$P4: A_{samp} = [\left(A_{GSD}{}^{P4}\ x\ T_{sec}{}^{P4}\right)\ x\ 0.9]$$

where M2 and P4 refer to values for the specific drone unit and T_{sec} is the average time (in seconds) for each video recorded (M2; one video per event) or flight duration (P4).

"Number of stages" (N_{stages}) were calculated as total count of stages relevant to each protocol from initial decision to use the protocol in a given project (e.g., planning, stage 1) leading to confirmation of detection (stage 10). Counts for all protocols included initial stages of analyses (e.g., planning, preparation), though some middling stages were not included for a given protocol (e.g., BAVS did not include sample processing or shipment).

Cost sub-categories

For each Cost sub-category, values were calculated using the considerations and assumptions outlined in Table 10. For all cost calculations, project-specific travel expenses, fuel, vehicle mileage, purchase of capital equipment not related to protocol start-up, implementation, or daily-use are not included. For all sub-categories, protocols with the lowest calculated values were considered "best" (e.g., rank = 1) while highest values were considered worst (e.g., rank = 14).

"Start-up costs" (C_{start}) are estimated based on: 1) fees for permit(s), certifications(s), and/or equipment registration specific to initial implementation of protocol (C_{fee} in equation below); 2) base equipment costs, including initial purchase of daily-use field equipment or cost of unit(s) necessary for conducting protocol (C_{equip} in equation below), and 3) costs associated with accessories and/or specialized software (C_{acc} in equation below) using the following formula:

Equation 16

$$C_{start} = \sum\left(C_{fee1} + C_{fee2} + \ \dots\right) + \left(C_{equip1} + C_{equip2} + \ \dots\right) + \left(C_{acc1} + C_{acc2} + \ \dots\right)$$

"Cost per event" (C_{event}) is estimated based on: 1) consumable equipment or supply costs per sample (C_{con} in equation below); 2) contracted (analyses or collection) costs per sample (we used contracted costs from this study) (C_{lab} in equation below); and 3) estimated salary for personnel conducting activities associated with protocol (C_{pers} in equation below) using the following formula:

Equation 17

$$C_{event} = \sum(C_{con1} + C_{con2} + \ldots) + (C_{lab1} + C_{lab2} + \ldots) + (C_{pers})$$

where C_{con} calculations can include, but are not limited to, sample collection supplies (bottles, bags, filters, gloves, sample containment vessels, etc.), paper for datasheets or forms, writing utensils, etc. Salary calculations (C_{pers}) were estimated based on the following formula:

Equation 18

$$C_{pers} = [(S_H \; x \; T_H) \; x \; T_{Tot}] \; x \; N_{pers}$$

where S_H is estimated hourly salary, T_H is the total number of hours in a typical work day, T_{Tot} is the total amount of Time calculated from the pre-field, field, and post-field sub-categories (equations follow), and N_{pers} is the minimum number of personnel from Equation 1. For our calculations, S_H = \$15 per hour, T_H = 8 hours.

Table 10 Sub-categories, factors, considerations, assumptions, and ranking orders used in the Costs category of the protocol comparison rubric.

Sub-category, factor, *(scale)*	**Calculation considerations**	**Calculation assumptions**
Start-up costs C_{start} *(USD, \$)*	Includes calculations for one-time equipment or protocol specific costs (e.g., permit/certification/registration fees, base equipment costs, software/accessory costs); excludes cost of transportation source (vehicles, vessels), major laboratory equipment (e.g., qPCR machine), sample storage (e.g., -80C freezer, refrigerator).	Assumes transportation, major equipment, or sample storage costs are maintained as part of routine laboratory upkeep. Example estimates: \$54 for TPWD research permit; \$1,000 for eDNA sample processing equipment; \$500 for general field electronics (camera, GPS, range-finders, etc.); \$5,000 for detector dog training; \$1,000 for traps array/apparatus and/or supplies; initial drone unit purchase (specific to unit; includes battery costs); \$550 ArcGIS Online subscription.
Costs per event C_{event} *(USD, \$)*	Includes cost of consumable supplies or equipment that needs to be replenished monthly, quarterly, or yearly; also includes cost per sample for contracted services (e.g., eDNA, CSS); does not include travel.	Assumes upkeep and general maintenance of field equipment as part of routine laboratory upkeep. Example estimates: \$5 per sample for general items (paper, writing utensils, etc.); contracted charges for eDNA sample analyses (\$40 per sample) or CSS field survey events (\$500 per event), estimated salary for associated protocol activities (e.g., \$15/day multiplied by cumulative number of hours from Time sub-categories)
Time (pre-field) T_{pre} *(days, #)*	Time required to prepare for field sampling; does not include time for preparation building up to field activities or season.	Assumes 8-hour day; includes typical day-to-day preparation. Examples include: gear preparation checklist, checking charge or charging of electronics, monitoring weather prior to field activities, etc.
Time (field) T_f *(days, #)*	Time required to conduct field activities related protocol.	Assumes maximum 6 hours of field activities per day; uses same values included in "Number of sites" calculation from Statistics category.
Time (post-field) T_{post} *(days, #)*	Time required for general post-field activities; does not include calculations for specific analyses related to research question(s)	Assumes 8-hour day. Examples include: equipment decontamination protocols, sample processing, data entry/QC, data compilation, etc.

"Time" for pre-field (T_{pre}), field (T_f), and post-field (T_{post}) activities were calculated as number of days. We used an 8-hour day for T_{pre} and T_{post} and a 6-hour day for T_f. Calculations for T_{pre} assumed a minimum of 2-hours (0.25 days) and was calculated using the following formula:

Equation 19

$$T_{pre} = \sum \frac{T_{hp}}{T_{od}}$$

where T_{hp} is the number of hours for preparation and T_{od} is the number of hours in a given office day. For our calculations, we used T_{od} = 8 hours. To calculate T_f, we used the inverse of Equation 2 resulting in:

Equation 20

$$T_f = \sum \frac{T_{sh}}{T_{fd}}$$

where T_{sh} and T_{fd} are the same values from Equation 2. To calculate T_{post}, we included: 1) average sample processing time calculated from results of this study (t_{samp}); 2) estimated shipping preparation time for batched samples sent to contracted labs (t_{ship}); 3) time for data entry and QC based on a per-site estimation (t_{entry}); 4) time for post-sampling data processing (e.g., video and/or imagery analyses) based on a per-site estimation ($t_{process}$); and 5) allowed an "other" variable for additional post-field related activities (e.g., advertisement of ORT) specific to a given protocol (t_{other}) in the following formula:

Equation 21

$$T_{post} = \sum (t_{samp} + t_{ship} + t_{entry} + t_{process} + t_{other})$$

Finally, total time for pre-, field, and post- activities (T_{Tot}) was calculated as:

Equation 22

$$\sum (T_{pre} + T_f + T_{post})$$

and included in Equation 18.

Online Reporting Tool Statistic and Cost category calculations

Calculations for the ORT followed similarly to those outlined above, with the following exceptions. "Number of personnel" included one person for development and maintenance of the tool and another for data QC, as "data entry" is conducted internally within the reporting tool. Because the ORT does not have a direct calculation for "Number of sites", the total number of reports made was divided by the number of weeks the tool was actively advertised and made available for reporting. "Geographic coverage" was assumed to be 100% as distribution of the reporting tool was sent to in- and out-of-state individuals and advertised through multiple channels. This distribution method allowed for anyone with familiarity of the greater east Texas area to be able to make a report and was not confined to a distinct spatial area (as with traditional field "sites"). "Detection proportion" was calculated as the number of photo-verified WCT reports divided by the total number of reports submitted to the reporting tool. For Time sub-categories, 10-days for preparation, approval, and development was used to for T_{pre}, 1-day for initial distribution and advertisement was used for T_f, and T_{post} assumed 1-day per month for continued advertisement and data management.

Comparison Rubric Tests

To test the functionality of the rubric, a series of hypothetical scenarios were applied. Scenario #1 represented a null test of the rubric where all subcategories were weighted equally (value = 1) and all protocols were included to determine an overall rank of "best recommendations", regardless of concerns for detection, cost, physical capture, etc. In Scenario #2, we adjusted sub-category weights to simulate the question "What is the best method to use if the goal of a project is to *capture* individuals *regardless of cost*?" Sub-categories were weighted between 0-1 with 0 = lowest priority and 1 = highest priority and only applied to methods that resulted in physical capture of WCT. In Scenario #3, we applied the same sub-category weights as in Scenario #2, but compared all methods resulting in WCT detections to simulate the question "What is the best method to use if the goal of a project is to *detect* individuals *regardless of cost*?". Finally, in Scenario #4, we adjusted sub-category weights to simulate the question "What is the best method to use if the goal of a project is to *detect* individuals with *limited funding*?" and used the same protocols from Scenario #3. Weights applied for each scenario can be found in Table 11.

Table 11 Weights used in scenarios applied to test protocol comparison rubric.

	Scenario #1	**Scenario #2**	**Scenario #3**	**Scenario #4**
Scenario objective:	No concern for detection or cost	Physical capture, regardless of cost	Detection, regardless of cost	Detection with concern for cost
Protocols applied to:	All protocols	CSS, Hoop Trap	All protocols, excluding WS	All protocols, excluding WS
Permissions	1.00	0.25	0.25	0.25
Planning	1.00	0.25	0.25	0.25
Difficulty of gear transport	1.00	0.00	0.00	0.75
Difficulty of implementation	1.00	0.00	0.00	0.75
Time and maintenance	1.00	0.00	0.00	0.75
Technical expertise	1.00	0.25	0.25	0.25
Performance variability	1.00	0.50	0.50	1.00
Potential for failure	1.00	0.50	0.50	1.00
Resolution	1.00	0.75	0.75	1.00
Number of personnel (N_{pers})	1.00	0.00	0.00	0.00
Number of sites (N_{sites})	1.00	0.25	0.25	0.25
Detection probability (ρ)	1.00	1.00	1.00	1.00
Geographic coverage (G_{cov})	1.00	0.50	0.50	0.50
Detection Proportion (Det%)	1.00	1.00	1.00	1.00
Stages of analysis (N_{stages})	1.00	0.00	0.00	1.00
Start-up costs (C_{start})	1.00	0.00	0.00	1.00
Cost per event (C_{event})	1.00	0.00	0.00	1.00
Time (pre-field) (T_{pre})	1.00	0.00	0.00	1.00
Time (field) (T_f)	1.00	0.00	0.00	1.00
Time (post-field) (T_{post})	1.00	0.00	0.00	1.00

Species Distribution and Habitat Modeling

Species distribution models (SDM) were conducted in MaxEnt (Phillips et al., 2020). Historic and recent WCT occurrence records were compiled from VertNet (http://vertnet.org/), iNaturalist (https://www.inaturalist.org/), publications (Adams and Saenz, 2011; Franklin et al., 2019; Ryberg et al., 2017), personal communications, and the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/). Across all occurrence sources, duplicate reports were removed and remaining locations were evaluated for geographic accuracy (see Appendix B for the full list of occurrences used in SDM analyses). Additionally, a list of confirmed occurrence locations resulting from the current study was compiled for use in SDM. Final models were run in three iterations: historic (occurrence data resulting from the year 2000 and earlier), current (occurrence data resulting after the year 2000 including potential and confirmed detections in the current study), and detection (using only confirmed detections from the current study).

Environmental co-variates were selected based on results of small-scale habitat analyses, previous SDM focused on WCT and other aquatic turtles, and previous assessments of anthropogenic impacts to aquatic turtles (Table 12) (Ryberg et al., 2017; Stratmann et al., 2016; Stryszowska et al., 2016; Kagayama et al., 2020). Data layers included: land cover from the U.S. Geological Survey (USGS) National Land Cover Database (NLCD; USGS, 2001, 2019), elevation from the USGS National Map (TNM) extracted as a 1 arc-second digital elevation model (DEM) (USGS, 2013), wetland boundaries from the USFWS National Wetlands Inventory (NWI; USFWS, 2022), soil types from the U.S. Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) Hydrologic Soil Groups (HSG; USDA, 2021), and road networks from the Texas Department of Transportation (TXDOT; TXDOT, 2023). Slope was calculated from the DEM to represent geographic obstacles or constraints to movement. Land cover type was included as raw values and as a “majority” value. Raw land cover data were included to account for features directly associated with WCT occurrences. Majority value was calculated within a specified neighborhood (using the estimated WCT home range = 703,114 m^2 from Bowers et al., 2021) using Focal Statistics to simulate the land cover types which may be encountered by WCT in their day-to-day movements. Euclidean distance between Freshwater Emergent and Pond wetland types from the NWI was determined as an indicator of wetland connectivity across the landscape. Road density (km/km^2) was calculated using Line Density with a search radius specified to the estimated WCT home range.

Table 12 Species distribution model (SDM) environmental co-variates, data source, model iteration(s) data were input to, layer name, and description of co-variate data extraction.

Co-variate	Data source	Model input	Layer name	Description
Distance between wetlands	NWI (2022)	All	wetlands_fwpe_eucdist_maxent	Euclidean distance between freshwater emergent and pond wetland types
Elevation	USGS (2013)	All	dem_tx_east_maxent	Raw topographic data from the digital elevation model (DEM)
Land cover class - majority	NLCD (2001)	Historic	nlcd_2001_majority_maxent	Most frequent landcover class type assigned to each cell within square neighborhood representative of home range (Bowers et al., 2021)
Land cover class - majority	NLCD (2019)	Current, detection	nlcd_2019_majority_maxent	Most frequent landcover class type assigned to each cell within square neighborhood representative of home range
Land cover class - raw	NLCD (2001)	Historic	nlcd_2001_east_maxent	Landcover class type present within each cell
Land cover class - raw	NLCD (2019)	Current, detection	nlcd_2019_east_maxent	Landcover class type present within each cell
Road density	TXDOT (2023)	All	txdot_rdwys_density_maxent	Calculated line density assigned to each cell within circular neighborhood representative of home range
Slope	USGS (2013)	All	dem_slope_east_maxent	Calculated slope within each cell based on elevation changes from the DEM
Soil class	USDA (2021)	All	soil_hsg_east_maxent	Hydrologic soil group present within each cell

Datasets were harmonized with occurrence locations in ArcGIS Pro prior to import into MaxEnt. All layers were projected to the Albers Conical Equal Area coordinate system for consistency. Layers were constrained to within counties of known historic range using the Clip or Extract by Mask tool (DOI, 2014). Jackson County was added to the county layer due to presence of a historic occurrence within the county boundary, though it is not considered part of the historic range for WCT. All data were converted to the ASCII file format and the historic, current, and detection occurrence tables were exported to .csv format for incorporation into MaxEnt. See Appendix E (electronic) for an ArcGIS Pro map package containing all finalized data used in SDM in addition to the MaxEnt ASCII layer outputs of the predicted distributions.

Data from NLCD 2001 were imported into the historic SDM while data from the NLCD 2019 were imported into the current and detection SDM. Data from the TNM, NWI, HSG, and TXDOT were assumed to remain constant over the entire period for which occurrence data were available (1922-2022) and used across all three SDM. All SDM were conducted with the following settings: 1) auto features enabled; 2) “create response curves”, “make pictures of predictions”, and “do jackknife to measure variable importance”, options selected; 3) output format = logistic; 4) "random seed” selected and “remove duplicates” deselected; 5) maximum number of randomized background points generated was kept at the default (10,000); and 6) models were run in five replicates using cross-validation. For the detection SDM, the default prevalence value was changed from 0.5 to 0.182 to more accurately indicate the detectability of WCT since the sampling methods were known. This was calculated by dividing the number of sampled sites with confirmed detections (n = 12) by the total number of sampled sites (n = 66).

RESULTS

Field Survey Results

From March 2020 through July 2022, 66 sites in 33 counties were sampled during the in-season sampling period resulting in 346 sampling events while four sites in four counties were sampled monthly between August 2021 through February 2022 during the out-of-season sampling period resulting in 28 sampling events (Figure 17; Tables 13 and 14). Minimum number of in-season sampling events at a site was four while maximum number of sampling events at a site was 12. Across all sites, survey areas ranged from 0.00–110.74 hectares (ha) of wetted habitat (average: 7.306 ± 15.0632 ha). One site (HC004) was dry during all sampling events due to localized drought conditions. Events on 24 May 2021, 25 May 2021, and 03 June 2021 were canceled due to inclement weather. Additionally, drone and CSS surveys were rescheduled or canceled due to inclement weather on 05 May 2021, 17 May 2021, and 13 July 2021.

Across all methods, during in-season sampling efforts, WCT presence was confirmed during 42 field survey events (12.1%) while potential presence was documented during 27 field survey events (7.8%). Out-of-season sampling resulted in 8 (28.6%) field survey events with confirmed WCT detections and two (7.1%) field survey events resulting in potential detections. In addition to in- and out-of-season field efforts, the ORT resulted in an additional 10 confirmed WCT reports not associated with field survey dates. Overall, a total of 102 confirmed WCT detections were made over the course of the study. In-season sampling efforts resulted in a total of 88 confirmed detections of WCT across all events, sites, and protocols while out-of-season efforts resulted in 14 confirmed detections of WCT across all events, sites, and protocols. A full list of other herpetofaunal species observed using each protocol can be found in Appendix F.

Across all survey methods (including photo-verified results from the ORT; Figure 18), WCT presence was confirmed at 12 locations in 10 counties including Austin, Brazoria, Chambers, Harris, Hopkins, Jasper, Jefferson, Nacogdoches, Upshur, and Waller. At locations with confirmed detections across all methods (including photo-verified reports via the ORT), WCT activity was documented in all calendar months except January and November. Potential presence (based on eDNA results) was documented in an additional 8 locations in seven counties including Anderson, Austin, Delta, Freestone, Harris, Hopkins, and Rockwall. Historic occurrence data downloaded for use in randomized site generation and SDM resulted in occurrences for an additional 10 counties including Brazos, Cass, Falls, Fort Bend, Hardin, Harrison, hill, Liberty, Milam, and Montgomery. A full list of historically occupied counties and updated occupancy status can be found in Appendix G.

Overall, eDNA samples resulted in 71 (7.6%) positive and 67 (7.2%) potential detections of WCT out of 935 samples analyzed. Overall, positive detections occurred at seven sites and potential detections at an additional eight. Presence of WCT was confirmed at three sites by photo-verified reports to the ORT in years 2 and 3, though one site yielded only potential eDNA results during field surveys and the others yielded no detections from any protocol. Additionally, during two events at different sites, soil samples were collected at locations where WCT were observed or captured. Both samples were collected from the approximate location where the turtle was originally found, yet both resulted in no amplifications of WCT DNA.

A total of 417 ambient water eDNA samples from 65 sites resulting in 35 positive detections (9.5%) at seven sites and 31 potential detections (7.4%) at 13 sites. Ambient water samples were filtered two pore sizes: 0.45 µm (“A-0.45”) and 3.0 µm (A-3.0). A total of 48 A-0.45 samples from four sites resulted in seven positive detections (14.6%) at all sites and seven potential detections (14.6%) at three sites. A total of 369 A-3.0 samples from 65 sites resulted in 28 positive detections (7.6%) at seven sites and 24 potential detections (6.5%) at 12 sites. A total of 418 resuspended sediment samples from 65 sites resulting in 34 positive detections (8.1%) at seven sites and 31 potential detections (7.4%) at 10 sites. As with ambient water samples, resuspended sediment samples were filtered using two pore sizes (R-0.45 and R-3.0, respectively). A total of 50 R-0.45 samples from five sites resulted in six positive detections (12.0%) at four sites and 10 potential detections (20.0%) at three sites. A total of 368 R-3.0 samples from 65 sites resulted in 28 positive detections (7.6%) at 10 sites and 21 potential detections (5.7%) at 10 sites. Overall, 100 soil samples from 10 sites resulted in two positive detections (12.0%) at two sites and four potential detections (8.0%) at three sites.

Across all years, 5,399 minutes of WS were conducted at 37 sites over 178 events. Average WS duration for each event was 30.3 ± 0.64 minutes, but varied between sites depending on how many field personnel were present (range: 20-121 minutes). Overall, WS were the only protocol applied which resulted in zero WCT detections.

Across all years, 18,966 minutes of BAVS were performed. Average BAVS duration for each event was 28.84 ± 0.35 minutes, but varied between sites depending on how many field personnel were present (range: 31-706 minutes). Ultimately, BAVS resulted in 10 observations (with a confidence level = 3) of WCT at two sites. Additionally, there were four potential WCT detections resulting in confidence levels < 3, though the turtles in question were too far away for species identification to be confidently confirmed.

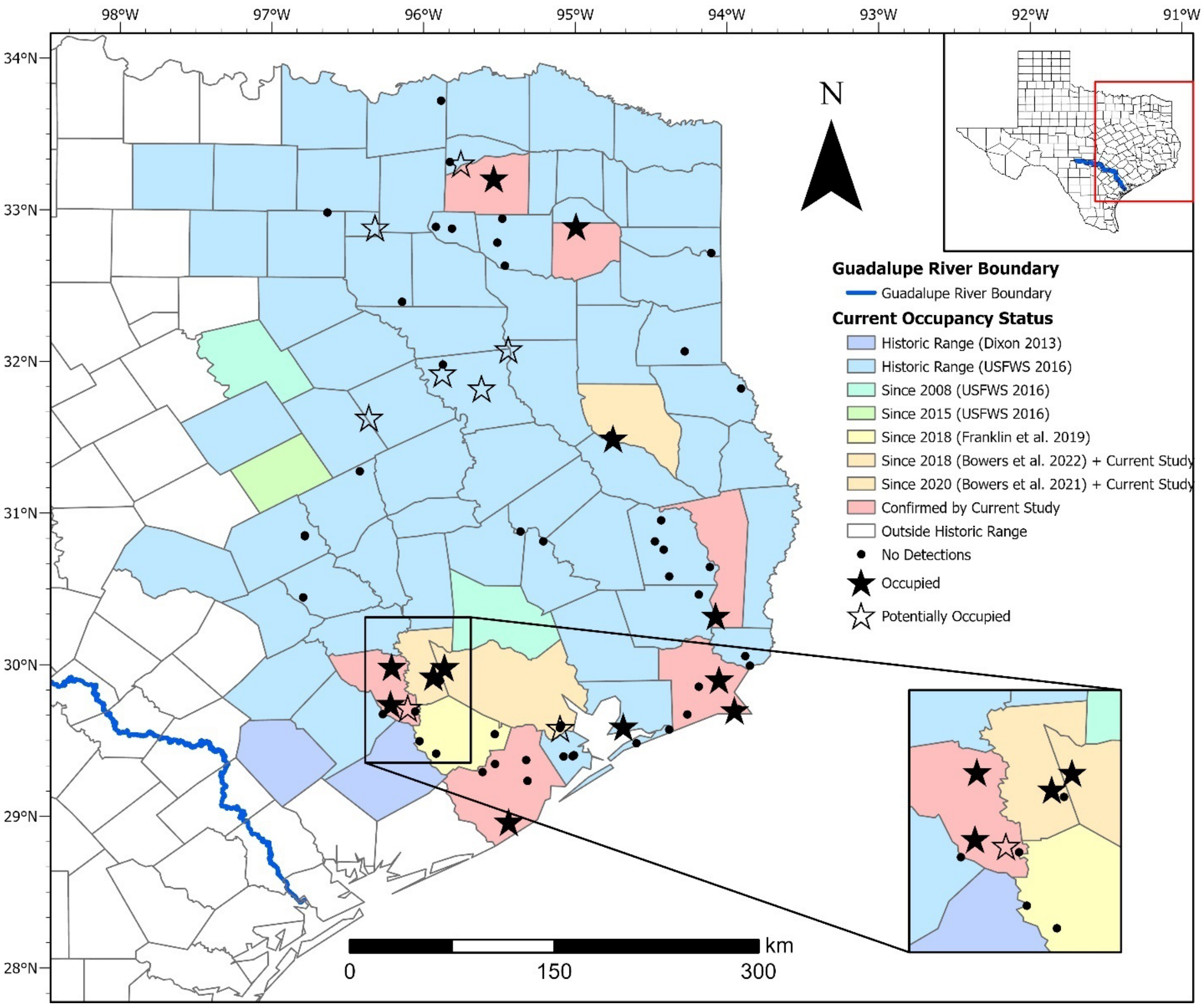


Figure 17 Confirmed occupancy (black stars; *n* = 12), potential occupancy (hollow stars; *n* = 8), and no detection (black points; *n* = 48) for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) surveys in east Texas. Historic range derived from Dixon (2013), USFWS (2016), Franklin et al. (2019), and Bowers et al. (2021, 2022a). County colors correlate to age of last established occupancy, e.g., cooler colors equate to older known counties of occupancy while warmer colors equate to counties with more recently established or confirmed occupancy. Counties with confirmed occupancy from the current study are indicated by the warmest colors.

Table 13 Sites sampled during the "in-season" sampling period (March-July). Wetland classification determined from overlapping National Wetland Inventory (NWI) category or nearest geomorphologically similar classification if no overlapping boundary was present. Wetted survey area calculated as total wetted area surveyed around assessment point. Status determined from cumulative results of all protocols employed (Occupied = at least one confirmed detection via any protocol; Potentially Occupied = potential detection from eDNA protocols; No Detections = no confirmed detections via any protocol). Cumulative number of environmental DNA samples (eDNA; all five matrix types), static images (multispectral drone [P4] includes counts for all spectra; game camera [GC] includes total number of images captured), and effort (in minutes) for binocular assisted visual surveys (BAVS), canid scent surveys (CSS), drone video (M2), walking surveys (WS), and hoop trap surveys (Hoop) are presented. Effort data for the online reporting tool (ORT) or road surveys (RS) are not included as they do not have specific site associations.

Site Type	Site ID	County	Wetland classification	Wetted Area (ha)	Status	eDNA *Samples*	P4 *Images*	GC	BAVS	CSS	M2 *Time (min)*	WS	Hoop
Control	CON01	Nacogdoches	Emergent	2.38	Occupied	59[†]	-	-	732[†]	215[†]	-	396	-[†]
	CON02a	Waller	Emergent	0.51	Occupied	60[†]	-	-	449	-	-	174	-[†]
	CON02b	Harris	Riverine	1.50	Occupied	60[†]	-	-	627	-	-	124	-[†]
	CON03	Chambers	Pond	0.81	Occupied	66[†]	8,316	-	739	1085[†]	354[†]	104	-[†]
Historic	HA001	Jefferson	Estuarine	0.25	No Detections	10	-	-	250	-	-	1	-
	HA003	Delta	Pond	110.74	Potentially Occupied	16	-	-	392	-	-	277	-
	HA011	Anderson	Forest/Shrub	5.37	Potentially Occupied	16	8,178	-	653	165	259	280	30,243
	HA013	Wharton	Forest/Shrub	32.33	No Detections	4	-	-	66	-	-	189	-
	HA015	Hardin	Pond	0.07	No Detections	8	-	-	153	-	-	13	-
	HA017	Delta	Riverine	0.20	No Detections	8	-	-	178	-	-	75	-
	HA020	Rockwall	Pond	0.06	Potentially Occupied	16	-	-	333	-	-	58	-
	HA021	Harrison	Pond	100.00	No Detections	8	-	-	243	-	-	37	-
	HA022	Robertson	Pond	2.91	No Detections	8	-	-	208	-	-	187	-
	HA024	Galveston	Pond	3.21	No Detections	8	-	-	166	-	-	-	-
	HA026	Tyler	Pond	0.18	No Detections	8	-	-	160	-	-	-	-
	HA029	Galveston	Pond	0.58	No Detections	8	-	-	162	-	-	-	-
	HA032	Brazoria	Pond	0.46	No Detections	8	-	-	238	-	-	5	-
	HA034	Galveston	Lake	38.97	No Detections	8	-	-	143	-	-	20	-
	HA047	Brazoria	Pond	0.10	No Detections	8	-	-	223	-	-	-	-
	HA048	Fort Bend	Emergent	0.28	No Detections	6	-	-	80	-	-	124	-
	HA050	Kaufman	Emergent	35.71	No Detections	8	-	-	234	-	-	-	-
	HA051	Galveston	Emergent	0.05	No Detections	8	-	-	158	-	-	10	-
	HA264	Jefferson	Riverine	11.36	No Detections	8	-	-	187	-	-	-	-
New	HC001	Galveston	Estuarine	13.17	No Detections	8	-	-	167	-	-	23	-
	HC004[a]	Milam	Emergent	0.00	No Detections	4	-	-	51	-	-	157	-
	HC005	Jefferson	Emergent	0.03	No Detections	6	-	-	159	-	-	35	-
	HC006	Austin	Forest/Shrub	0.18	Potentially Occupied	10	-	-	81	-	-	341	-
	HC008	Burleson	Pond	0.26	No Detections	8	-	-	180	-	-	1	-
	HC012	Freestone	Pond	0.05	Potentially Occupied	8	-	-	107	-	-	30	-
	HC013	Brazoria	Pond	12.24	No Detections	8	-	-	367	-	-	73	-

Table 13 Sites sampled during the "in-season" sampling period (March-July). Wetland classification determined from overlapping National Wetland Inventory (NWI) category or nearest geomorphologically similar classification if no overlapping boundary was present. Wetted survey area calculated as total wetted area surveyed around assessment point. Status determined from cumulative results of all protocols employed (Occupied = at least one confirmed detection via any protocol; Potentially Occupied = potential detection from eDNA protocols; No Detections = no confirmed detections via any protocol). Cumulative number of environmental DNA samples (eDNA; all five matrix types), static images (multispectral drone [P4] includes counts for all spectra; game camera [GC] includes total number of images captured), and effort (in minutes) for binocular assisted visual surveys (BAVS), canid scent surveys (CSS), drone video (M2), walking surveys (WS), and hoop trap surveys (Hoop) are presented. Effort data for the online reporting tool (ORT) or road surveys (RS) are not included as they do not have specific site associations.

Site Type	Site ID	County	Wetland classification	Wetted Area (ha)	Status	eDNA	P4	GC	BAVS	CSS	M2	WS	Hoop
						Samples	*Images*		*Time (min)*				
	HC016	Anderson	Lake	2.39	Potentially Occupied	16	-	-	491	-	-	-	-
	HC025	San Jacinto	Emergent	2.30	No Detections	8	-	-	203	-	-	128	-
	HC026	Rains	Pond	0.17	No Detections	8	-	-	70	-	-	279	-
	HC099	Dallas	Pond	0.76	No Detections	8	-	-	166	-	-	20	-
	HC171	Brazoria	Riverine	0.32	No Detections	8	-	-	217	-	-	-	-
	NC003	Panola	Emergent	6.97	No Detections	8		-	231	-	-	32	-
	NC010	Upshur	Pond	1.04	Occupied	$17^{\dagger}$	4,446	50,041	778	-	166	-	$30,165^{\dagger}$
	NC016	Trinity	Lake	11.98	No Detections	8	-	-	108	-	-	-	-
	NC020	Orange	Estuarine	1.00	No Detections	8	-	-	252	-	-	-	-
	NC094	Fannin	Pond	0.06	No Detections	8	-	-	137	-	-	172	-
	NC099	Wood	Pond	0.38	No Detections	8	-	-	188	-	-	84	-
Opportunistic	OPP02	Jefferson	Pond	0.45	Occupied	$32^{\dagger}$	-	150,648	$834^{\dagger}$	-	-	86	-
	OPP07	Tyler	Pond	0.23	No Detections	8	-	-	169	-	-	-	-
	OPP08	Tyler	Lake	1.50	No Detections	8	-	-	165	-	-	-	-
	OPP10	Tyler	Pond	1.01	No Detections	8	-	-	237	-	-	20	-
	OPP11	Anderson	Riverine	2.12	Potentially Occupied	16	6,900	-	836	-	228	258	31,188
	OPP13	Austin	Pond	0.32	No Detections	8	-	-	272	-	-	32	-
	OPP16	Tyler	Pond	2.35	No Detections	8	-	-	235	-	-	1	-
	OPP19	Milam	Riverine	4.46	No Detections	8	-	-	161	-	-	-	-
	OPP20	Harris	Pond	0.13	No Detections	10	-	-	235	-	-	-	-
	OPP21	Harris	Estuarine	2.57	No Detections	8	-	-	98	-	-	-	-
	OPP22	Fort Bend	Forest/Shrub	10.99	No Detections	10	-	-	221	-	-	92	-
	OPP23	Waller	Other	12.00	No Detections	16	10,794	-	683	-	437	43	
	OPP24	Jefferson	Pond	0.18	Occupied	$16^{\dagger}$	$1,236^{\dagger}$	$34,558^{\dagger}$	$682^{\dagger}$	-	162	317	$32,238^{\dagger}$
	OPP26	Anderson	Forest/Shrub	15.53	No Detections	8	-	-	340	-	-	95	-
	OPP32	Colorado	Pond	4.09	No Detections	8	-	-	286	-	-	-	-
	OPP33	Wood	Forest/Shrub	5.30	No Detections	8	-	-	423	-	-	-	-
	OPP34	Harris	Pond	0.65	Potentially Occupied	18	-	-	515	-	-	-	-
	OPP35	Nacogdoches	Forest/Shrub	1.55	No Detections	8	-	-	23	-	-	405	-
	OPP37[b]	Hopkins	Pond	0.22	Occupied	16	4,584	-	405	961	234	212	30,627

Table 13 Sites sampled during the "in-season" sampling period (March-July). Wetland classification determined from overlapping National Wetland Inventory (NWI) category or nearest geomorphologically similar classification if no overlapping boundary was present. Wetted survey area calculated as total wetted area surveyed around assessment point. Status determined from cumulative results of all protocols employed (Occupied = at least one confirmed detection via any protocol; Potentially Occupied = potential detection from eDNA protocols; No Detections = no confirmed detections via any protocol). Cumulative number of environmental DNA samples (eDNA; all five matrix types), static images (multispectral drone [P4] includes counts for all spectra; game camera [GC] includes total number of images captured), and effort (in minutes) for binocular assisted visual surveys (BAVS), canid scent surveys (CSS), drone video (M2), walking surveys (WS), and hoop trap surveys (Hoop) are presented. Effort data for the online reporting tool (ORT) or road surveys (RS) are not included as they do not have specific site associations.

Site Type	Site ID	County	Wetland classification	Wetted Area (ha)	Status	eDNA	P4	GC	BAVS	CSS	M2	WS	Hoop
						Samples	*Images*				*Time (min)*		
	OPP38[b]	Austin	Pond	0.09	Occupied	8	-	-	83	-	-	205	-
	OPP39[b]	Brazoria	Forest/Shrub	1.13	Occupied	5	-	-	83	-	-	234	-
Supp.	SRA01	Rains	Lake	1.60	No Detections	8	486	-	280	-	106	-	-
Supp.	SRA02	Wood	Forest/Shrub	6.13	No Detections	8	5,574	-	322	-	245	-	-
Supp.	SRA03	Shelby	Lake	4.85	No Detections	8	810	-	288	-	170	-	-
Supp.	SRA04	Orange	Riverine	1.40	No Detections	8	5,766	-	261	-	129	-	-

[a]No water present during sampling efforts due to localized drought conditions.
[b]WCT activity confirmed by report(s) to Online Reporting Tool, no positive detections during field surveys via any protocols.
†Indicates protocol resulting in confirmed detection of WCT. For hoop trap surveys with no associated effort, occupancy confirmed by collaborators trapping in sample area.

Table 14 Sites sampled during the "out-of-season" sampling period (August 2021-February 2022). Cumulative number of environmental DNA samples (eDNA; all five matrix types) and effort (in minutes) for binocular assisted visual surveys (BAVS). Methods resulting in positive detection of WCT indicated by "†".

Site ID	WCT Detected?	eDNA	BAVS
		# Samples	*Time (min)*
CON02a	No	21	163
CON02b	Yes	21†	191
CON03	Yes	21†	210
OPP02	Yes	23†	218†

Figure 18 Examples of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) captured or observed using field methods applied in the current study and photo-verified reports from the Online Reporting Tool (ORT). Top left: basking WCT observed during binocular assisted visual surveys (BAVS) (photo credit: J. Welch). Top right: live WCT detected and captured during a CSS (photo credit: D. DeChellis). Middle left: swimming WCT observed during drone surveys using the Mavic 2 Enterprise Dual (Drone$_{M2}$). Middle right: WCT found crossing the road and reported to the ORT (photo credit: T. Bowman). Bottom left: WCT found crossing a drag strip and reported to the ORT (photo credit: B. Pachar). Bottom right: WCT observed on basking platform installed in view of game camera.

Drone surveys were conducted at 11 sites in years 2 and 3 resulting in 96 surveys for both the $Drone_{M2}$ and $Drone_{P4}$. Between both platforms, a total of 57,090 static images ($Drone_{P4}$; average: 5,190.0 ± 1,006.21; range: 486-10,974 images) and 2,464 minutes of video imagery ($Drone_{M2}$; average: 226.5 ± 29.70; range: 107-354 minutes) were collected. Overall, drone surveys resulted in six WCT detections at two sites during six surveys (6.3%) (Figures 19 and 20). Five WCT were detected with the $Drone_{M2}$ whereas the $Drone_{P4}$ detected one WCT.

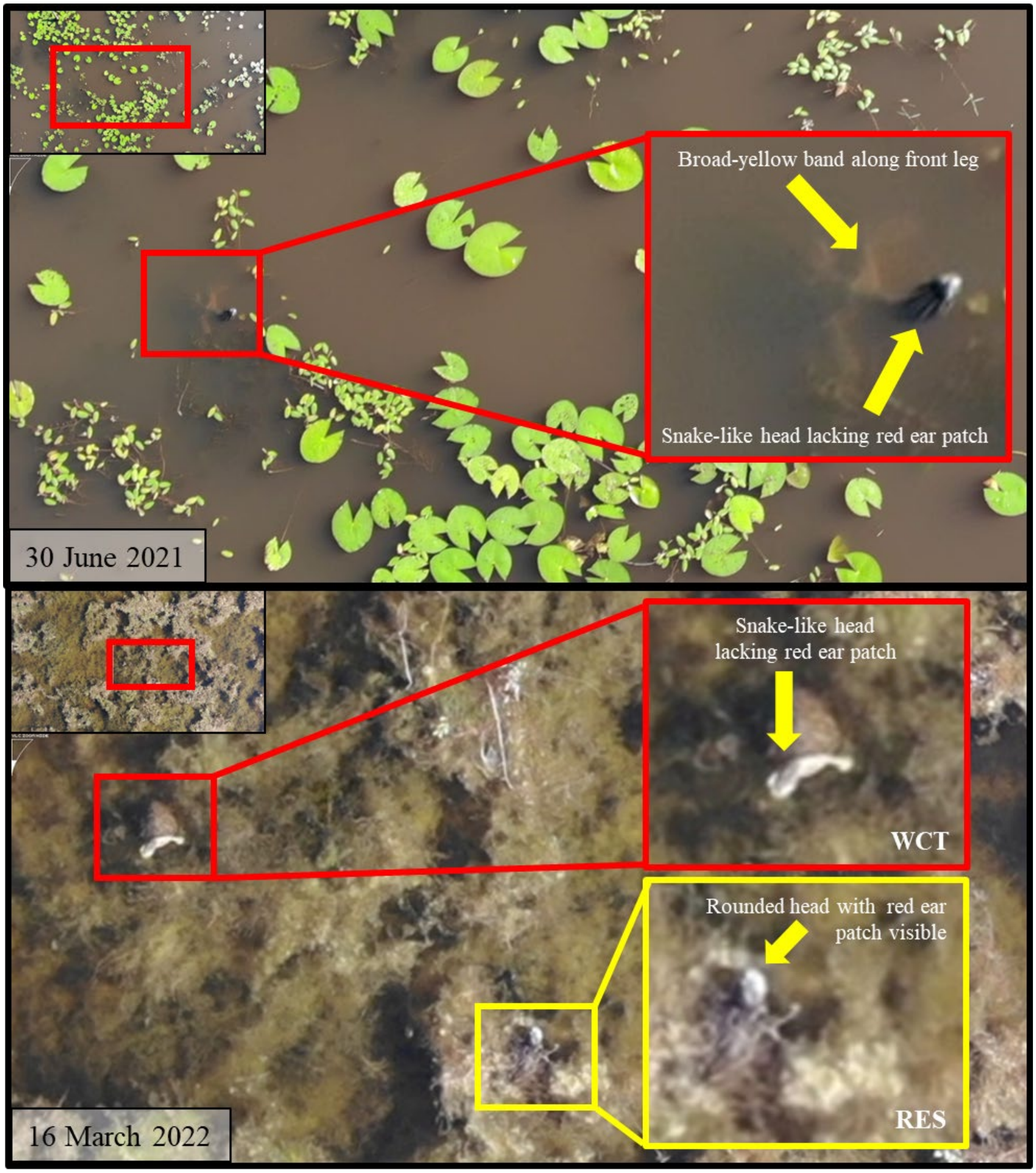


Figure 19 Confirmed Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) observations on two different dates using the Mavic 2 drone platform ($Drone_{M2}$). Top: WCT observed on 30 June 2021 with diagnostic traits used to confirm species identification highlighted. Bottom: comparison of diagnostic traits between a WCT observed in close proximity to a Red-eared Slider (*Trachemys scripta elegans*) on 16 March 2022 with traits used to confirm species identification highlighted.

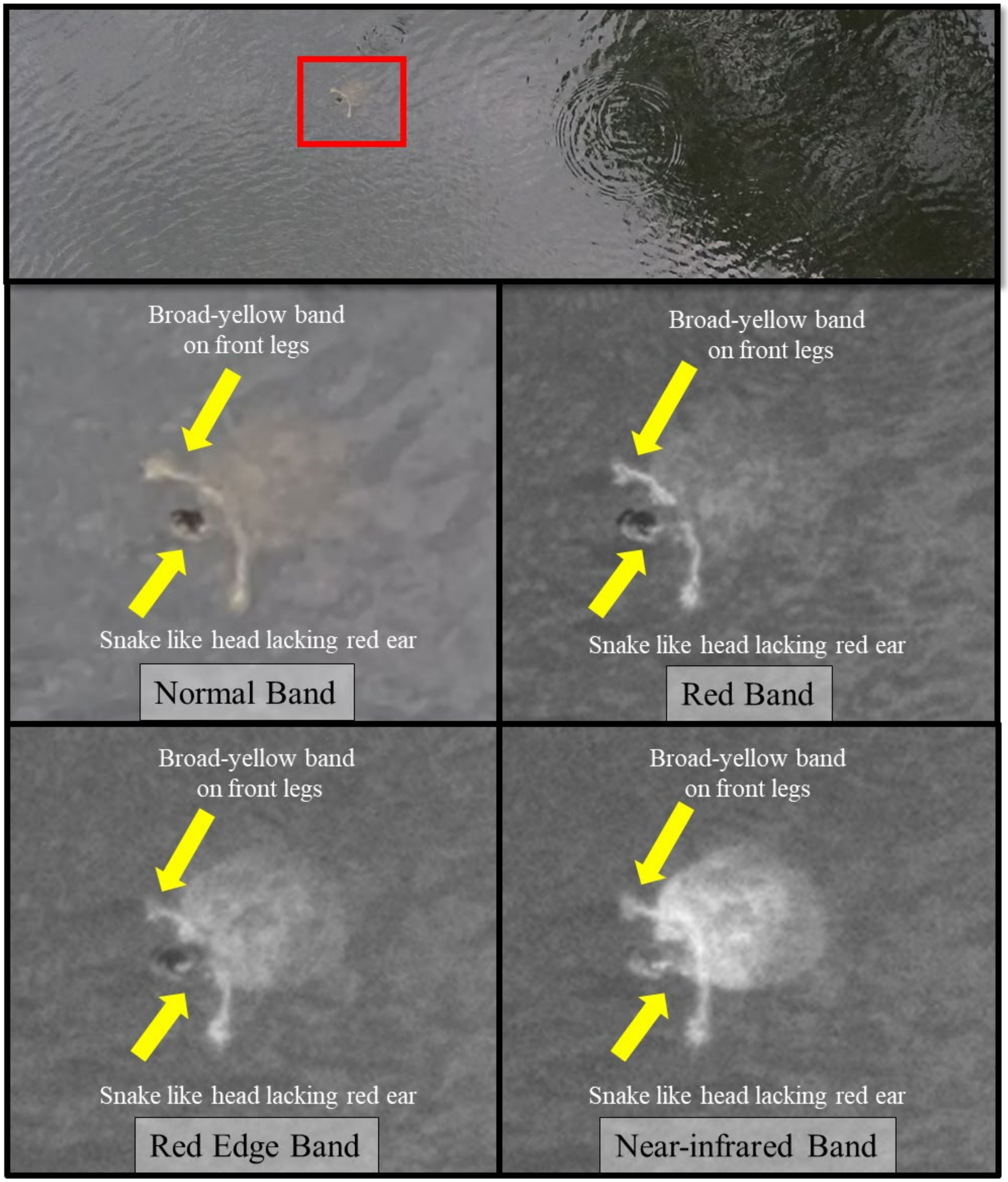


Figure 20 Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) observed using multispectral imagery from the Phantom 4 drone platform ($Drone_{P4}$). Top: overview of WCT breaching at water's surface in open water habitat. Middle left, middle right, bottom left, and bottom right: images of the same individual from the top image displayed in normal, red, red edge, and near-infrared bands (respectively) highlighting key diagnostic characteristics used for species identification. In this example, diagnostic characteristics used for identification were clearest in the normal and red bands.

From March 2021 through July 2022, game cameras were installed at one site because of WCT observations made in year 1 of the study. From March 2022 through July 2022, game cameras were installed at two additional sites because of WCT observations made in year 2 of the study. In total, game camera surveys resulted in 235,247 images (average: 78,415.7 ± 36,391.7, range: 34,558-150,648) collected during deployment. Overall, game cameras resulted in three observations of WCT at one site.

Hoop trap surveys were conducted at five sites during year 3 resulting in 20 events over a combined 107 trap nights. Two events (10%) resulted in capturing WCT. Average soak time for each event was 42.0 ± 0.39 hours (range: 40.9-48.1 hours). Trapping efforts resulted in three WCT captures at two sites. Hoop trap surveys were not performed at control sites due to concerns about impacts to an ongoing WCT population assessment being conducted by TPWD staff and researchers from another university at those sites. These researchers confirmed that WCT were captured during all trapping events occurring during or around the time of our field efforts for other methods (e.g., eDNA, drone, etc.), though their trapping efforts were focused on the middle period of our in-season sampling window (e.g., April and May).

Overall, a total of 23 CSS were conducted at four sites resulting in two WCT captures (8.7%). Across all years, a total of 2,426 minutes (average: 105.5 ± 8.20 minutes; range: 23-165 minutes) of CSS surveys were conducted. In year 1, preliminary CSS were conducted at one site in May and June to test the applicability of the method. One dead WCT was detected and found by the dog at this site in May 2020. This site was abandoned in years 2 and 3 due to concerns about the overall impact of the detector dog to an ongoing WCT population assessment being conducted by researchers from another university actively sampling at that site. In years 2 and 3, CSS efforts were conducted at three other sites during the in-season period. At one of these sites, TPWD staff confirmed capture of WCT in hoop trap surveys during all CSS survey dates in April and May. At another site, further CSS were abandoned after one survey due to safety concerns for the dog regarding presence of feral hogs (*Sus scrofa*) and alligators (*Alligator mississippiensis*). One WCT was detected and found alive by the detector dog in May 2021. In an attempt to increase detection potential at both sites, a second detector dog was added to survey efforts in year 3, though ultimately, no WCT were detected during CSS in year 3.

In addition to field surveys, opportunistic road surveys were conducted primarily between sampling locations as conditions allowed. A total of 53 surveys resulted in two observations of WCT (3.6%), both in Chambers county in areas surrounding one occupied site sampled using other protocols throughout the study period. Across all years of the study, 2,104 minutes of road surveys were performed covering 645.9 miles. Average survey duration was 39.7 ± 2.62 minutes (range: 6–101 minutes) over an average of 12.18 ± 1.508 miles per survey (range: 0.4-59.0). Average speed of all surveys was variable (range = 5-60 miles per hour), but most surveys were performed around 28.7 ± 2.11 mph. Road surveys were conducted in a variety of habitat types including: industrial, residential, agricultural, forested, urban, and parks.

Western Chicken Turtle (WCT) Capture Results

Across all years, six WCT (female: n = 4, male: n = 2,) were physically captured at five sites representing four counties (Nacogdoches: n = 1, Chambers: n = 1, Jefferson: n = 3, and Upshur: n = 1). Three methods yielded captures of WCT (CSS: n = 2, hoop trap: n = 3, opportunistic hand capture after BAVS: n = 1). No significant differences were detected for midline straight carapace length, maximum shell width, maximum shell depth, midline plastron length, or weight, though significant differences were detected between sexes for plastron width ($F_{1,4} = 14.712$, $p = 0.019$). Though data are insufficient for proper statistical analyses, clear differences between morphometric values for males and females were observed (Figure 21; Table 15). Two female WCT were examined with ultrasonography for presence of reproductive structures. One female was examined in May 2021 with a maximum follicle size of 1.96 mm and another in March 2022 with a maximum follicle size of 1.78 mm. Neither females exhibited eggs.

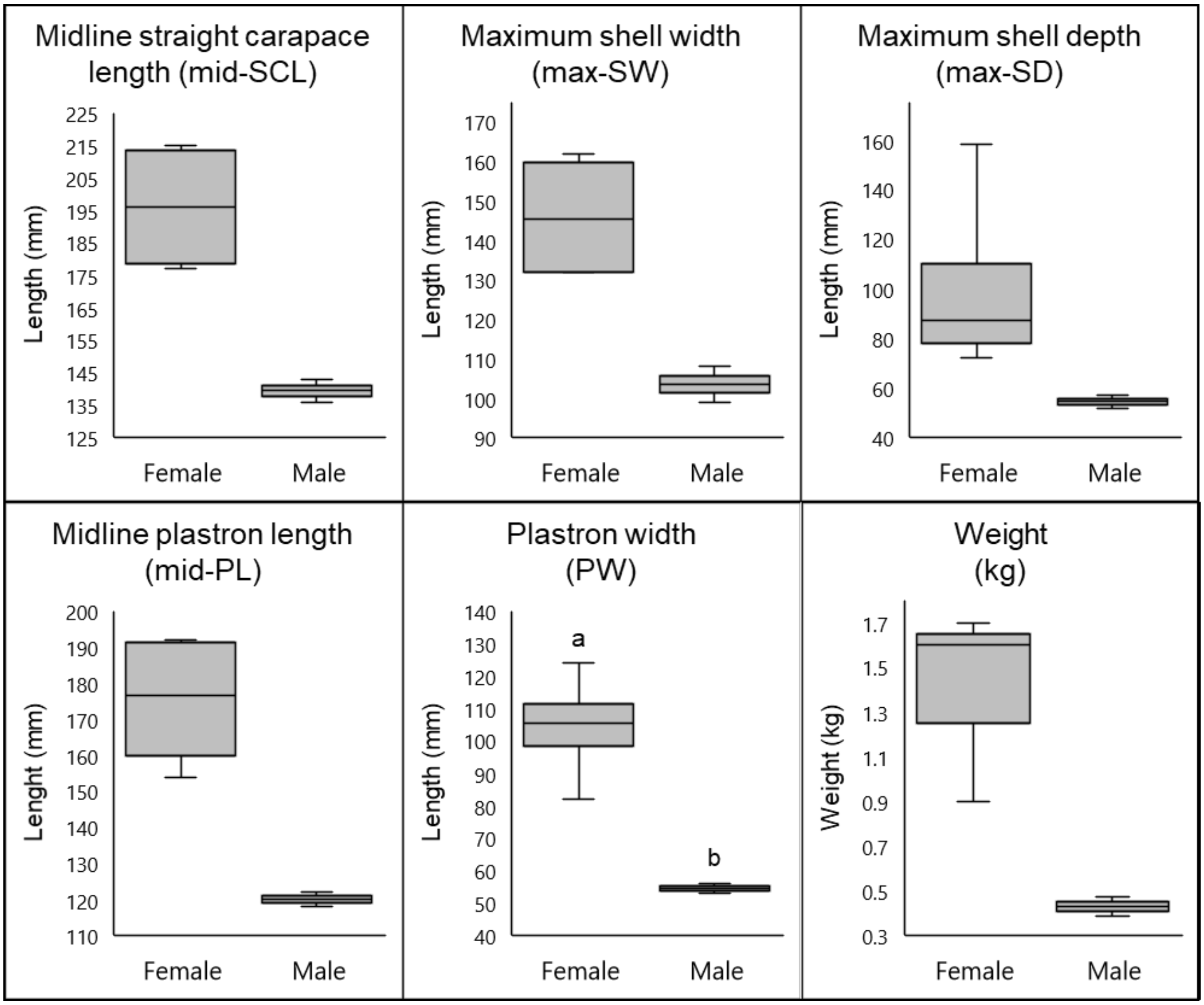


Figure 21 Boxplots of morphometric measurements for female ($n = 4$) and male ($n = 2$) Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) captured during the current study. No significant differences were detected for midline straight carapace length (mid-SCL; H = 3.429, df = 1, $p = 0.133$), maximum shell width (max-SW; H = 3.529, df = 1, $p = 0.133$), maximum shell depth (max-SD; $F_{1,4} = 2.511$, $p = 0.188$), midline plastron length (mid-PL; H = 3.429, df = 1, $p = 0.133$), or weight ($F_{1,3} = 8.784$, $p = 0.059$). Significant differences were detected between sexes for plastron width (PW; $F_{1,4} = 14.712$, $p = 0.019$). Statistically significant groupings indicated by letters above boxplots. Though data are insufficient for proper statistical analyses, clear differences between morphometric values for males and females are indicated.

Table 15 Morphometric values for female (F) and male (M) Western Chicken Turtles (WCT, *Deirochelys reticularia miaria*) captured during the current study. Includes measurements (in mm) for midline straight carapace length (mid-SCL), maximum shell width (max-SW), maximum shell depth (max-SD), midline plastron length (mid-PL), plastron width (PW), and weight (in kg). Average ± 1 standard error (SE) are presented for each sex.

ID	D01[a]	TPWD15	N001	N004	N002	N003	Average ± 1SE	
Capture Date	05/14/20	05/26/21	03/14/22	07/07/22	06/13/22	07/07/22	**F** (*n* = 4)	**M** (*n* = 2)
Sex	F	F	F	F	M	M		
Mid-SCL (mm)	179	177	215	213	136	143	196.0 ± 10.41	139.5 ± 3.50
Max-SW (mm)	132	132	159	162	99	108	146.3 ± 8.25	103.5 ± 4.50
Max-SD (mm)	80	72	94	158	52	57	101.0 ± 19.54	54.5 ± 2.50
Mid-PL (mm)	162	154	192	191	118	122	174.8 ± 9.81	120.0 ± 2.00
PW (mm)	107	104	124	82	56	56	104.3 ± 8.63	54.5 ± 1.50
Weight (kg)	--	0.90	1.60	1.70	0.39	0.48	1.40 ± 0.218	0.43 ± 0.043

[a]Found dead; no associated weight measurement.

Online Reporting Tool (ORT) Report Results

Links and reminders about the ORT were distributed to a combined total of 1,419 individuals or organizations (29 January 2021: *n* = 418; 04 May 2021: *n* = 492: 09 March 2022: *n* = 509) using an email distribution list. Additionally, the ORT was shared at multiple professional and agency meetings, highlighted in various newsletters and media outlets, and shared on social media (including Facebook and Instagram). Overall, the ORT was shared 153 times after social media posts were made.

Between January 2021 and April 2022, we received 49 responses to the ORT. Of the total responses, 23 (46.9%) respondents reported that they have never seen a WCT. Three responses (6.1%) included photographs of the individual observed allowing us to confirm that the individuals being reported were melanistic Red-eared Sliders (*Trachemy scripta elegans*). Of the remaining reports, 10 (20.4%) included photographs of WCT allowing us to confirm proper identification and reporting. All WCT reported were alive. One verified WCT report was received early enough that the location was used as a sample site in years 2 and 3 of the survey while two others were received early enough that those locations could be used as sample sites in year 3 of the study. All three sites added to the study design based on photo-verified reports of WCT to the ORT resulted in no confirmed detections during field survey efforts for any protocol. One site (added in year 2) returned potential eDNA detections, but no confirmed detections. Five reports originated from locations already included in the study design. Photo-verified observations occurred during 2018–2022 in March (*n* = 2), April (*n* = 4), May (*n* = 1), July (*n* = 1), October (*n* = 1), and December (*n* = 1). Of the respondents who did not provide a photograph but confirmed they were reporting observations of WCT (*n* = 13), 12 provided temporal data related to their observation (1971: *n* = 1; 1975: *n* = 1; 1978: *n* = 1; 2009: *n* = 1; 2015: *n* = 1; 2020: *n* = 6; and 2022: *n* = 1; April: *n* = 3; May: *n* = 1; June: *n* = 4; July: *n* = 3; August: *n* = 1).

Small-scale Habitat Analysis Results

Prior to small-scale habitat analyses, 28 (8.0%) events with status = 0.5 (potential detections) were removed to clearly define differences between detection and non-detection variables. Data from 322 events during the in-season period were used for small-scale habitat analyses. Data from the in-season period were used as these were the most consistently recorded across all years and sites and represented the least amount of temporal variation (when compared to out-of-season data). Overall variation in the data were tested by comparing detection versus non-detection results by site and event. Analyses by event produced the most significant interactions, thus, all small-scale habitat analyses are reported by event.

Initially, environmental and habitat variables observed during each in-season sampling event were compared regardless of wetland classification to determine if there were any significant correlations with WCT detections (Table 16). Specific conductivity (uS) was significantly lower during events where WCT were detected (p = 0.0014) (Figure 22) with the probability of detecting WCT predicted to reach zero if specific conductivity is greater than 1,470 uS (p = 0.0106). Overhead and ground cover percentage and types were also found to correlate with detection of WCT. Densiometer cover was significantly lower during events where WCT were detected (p = 0.0069) (Figure 23). Canopy cover in the middle height classification (0.5-5 m) was significantly lower at sites where WCT were detected (p = 0.0028) (Figure 24) with tall (> 0.5 m) grasses identified as the dominant cover type during the highest proportion of events where WCT were detected (0.317) (Table 17). Conversely, canopy cover in the lower height classification (< 0.5 m) was increased during events where WCT were detected, but not at the α = 0.5 level (p = 0.0823) (Figure 25) with grasses/herbs identified as the dominant cover type during the highest proportion of events where WCT were detected (0.690). Additionally, dominant ground cover type significantly differed between events where WCT were detected versus those where WCT were not detected (p = 0.0018). Pairwise comparison showed that detections of WCT were significantly higher during events with dominant ground cover categories including in-water vegetation (submerged aquatic vegetation [SAV] or floating aquatic vegetation [FAV]) compared to sites with a dominant ground cover type consisting of bareground or duff (p = 0.013) or grasses or herbs (p = 0.013).

Table 16 Summary of water quality and small-scale habitat variables for all sampling events and wetland types comparing events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected versus those where WCT were not detected. Values are presented as average ± 1 standard error (SE) with range (minimum to maximum) in parentheses. Significant differences based on WCT detections were tested using a Kruskal-Wallis One-Way ANOVA on Ranks. Significance (p-values) for each variable provided (α = 0.05) with significant p-values italicized.

Variable	WCT Not Detected	WCT Detected	Test Statistic	*p*-value
Water Temperature (°C)	25.65 ± 0.313 (9.9-38.0)	25.21 ± 0.811 (11.9-26.6)	H = 0.452	0.501
Specific Conductivity (µS)	660.75 ± 145.525 (4.8-22,492.0)	140.03 ± 15.758 (45.2-449.6)	H = 10.248	*0.001*
Dissolved Oxygen (mg/L)	6.101 ± 0.227 (0.24-18.88)	5.42 ± 0.609 (0.26-13.95)	H = 1.215	0.270
pH	7.418 ± 0.0559 (5.52-10.22)	7.141 ± 0.134 (5.73-10.13)	H = 2.937	0.087
Secchi (m)[a]	0.493 ± 0.0201 (0.01-1.00)	0.381 ± 0.0386 (0.01-1.00)	H = 3.504	0.061
Air Temperature (°C)	26.77 ± 0.317 (6.8-38.5)	25.96 ± 0.754 (12.2-33.9)	H = 1.290	0.256
Densiometer (%)	38.2 ± 2.33 (0-100)	23.1 ± 5.25 (0-93)	H = 7.301	*0.007*
Total Cover - Upper (%)	13.0 ± 1.48 (0-95)	10.9 ± 3.10 (0-70)	H = 2.647	0.104
Total Cover - Middle (%)	39.1 ± 1.45 (0-90)	27.7 ± 3.51 (0-85)	H = 8.945	*0.003*
Total Cover - Lower (%)	58.8 ± 1.20 (5-100)	64.4 ± 2.89 (19-90)	H = 3.020	0.082
# of Hydrology Indicators	7.7 ± 0.14 (0-14)	7.2 ± 0.33 (3-14)	H = 1.972	0.160
Total number of events (*n*)	280[b] (range: 258-280)	42	--	--

[a]Secchi maximum detection limit = 1.0 m; recorded as "> 1.000 m" if above detection limit
[b]Some parameters had < 280 events due to localized drought conditions at the site

Table 17 Proportion of events with and without Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) detections for categorical habitat variables. Categories variable with the highest proportion of confirmed WCT detections for each are italicized.

Variable	Category	Detections	No detections
Dom. cover - lower	*Grasses/Herbs*	*0.690*	*0.730*
Dom. cover - lower	SAV/FAV	0.286	0.094
Dom. cover - lower	Open Water	0.024	0.061
Dom. cover - lower	Bare/Duff	0.000	0.083
Dom. cover - lower	Trees/Shrubs/Vines	0.000	0.032
Dom. cover - middle	*Grasses*	*0.317*	*0.337*
Dom. cover - middle	Tree (D)	0.317	0.356
Dom. cover - middle	Shrubs	0.293	0.217
Dom. cover - middle	Herbs	0.073	0.079
Dom. cover - middle	Other	0.000	0.004
Dom. cover - middle	Tree (E)	0.000	0.007
Dom. cover - upper	*Tree (D)*	*1.000*	*0.894*
Dom. cover - upper	Tree (E)	0.000	0.106
NWI classification	*Freshwater Pond*	*0.571*	*0.432*
NWI classification	Freshwater Emergent	0.357	0.111
NWI classification	Riverine	0.071	0.129
NWI classification	Estuarine and Marine	0.000	0.057
NWI classification	Freshwater Forested/Shrub	0.000	0.146
NWI classification	Lake	0.000	0.096
NWI classification	Other	0.000	0.029
Observed land use	*Rural/Pasture*	*0.571*	*0.401*
Observed land use	Forest	0.429	0.430
Observed land use	Park	0.000	0.055
Observed land use	Resident	0.000	0.099
Observed land use	Urban	0.000	0.015
Observed wetland	*Emergent*	*0.476*	*0.260*
Observed wetland	Pond	0.476	0.378
Observed wetland	Forest/Shrub	0.048	0.087
Observed wetland	Estuarine	0.000	0.056
Observed wetland	Lake	0.000	0.149
Observed wetland	Riverine	0.000	0.069
Substrate - in water	*Clay*	*0.452*	*0.546*
Substrate - in water	Sand	0.405	0.207
Substrate - in water	Muck	0.095	0.151
Substrate - in water	Organic	0.048	0.085
Substrate - in water	Other	0.000	0.011
Substrate - shore	*Clay*	*0.524*	*0.613*
Substrate - shore	Sand	0.310	0.170
Substrate - shore	Muck	0.143	0.129
Substrate - shore	Organic	0.024	0.085
Substrate - shore	Mud	0.000	0.004

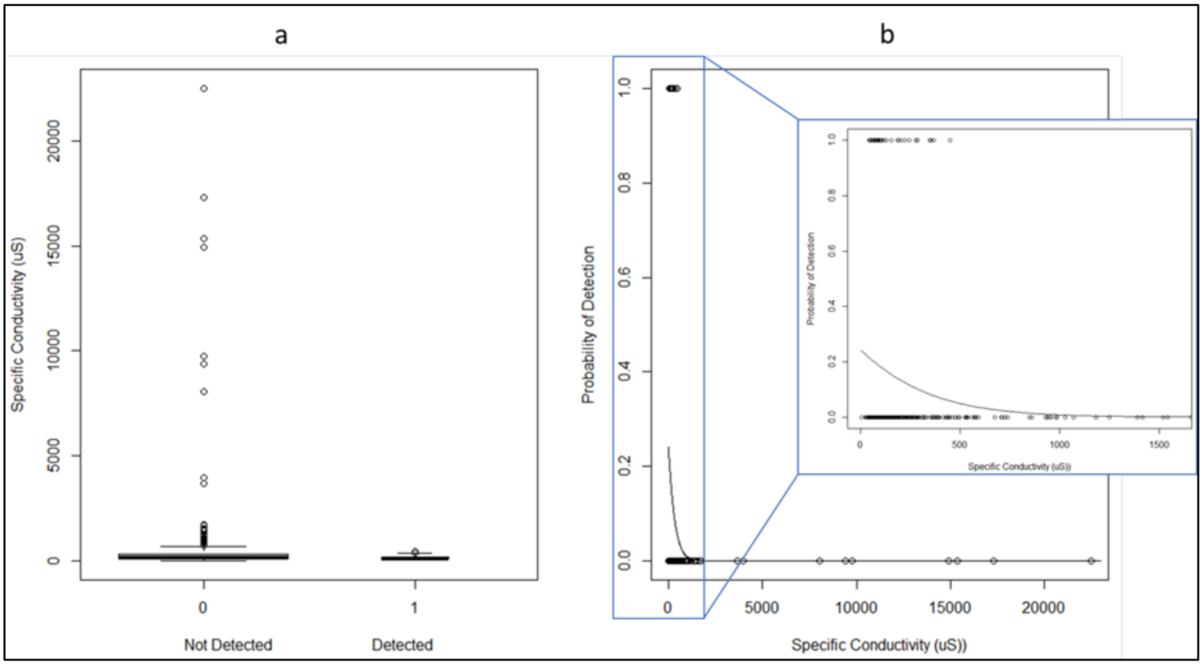


Figure 22 a) Boxplot of specific conductivity (uS) during events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected (1) versus not detected (0). Specific conductivity was significantly lower during events where WCT were detected (p = 0.0014). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of WCT by specific conductivity with detection probability curve (p = 0.0106). Based on results of the GLM, probability of detecting WCT is predicted to reach 0 if specific conductivity is greater than 1,470 µS.

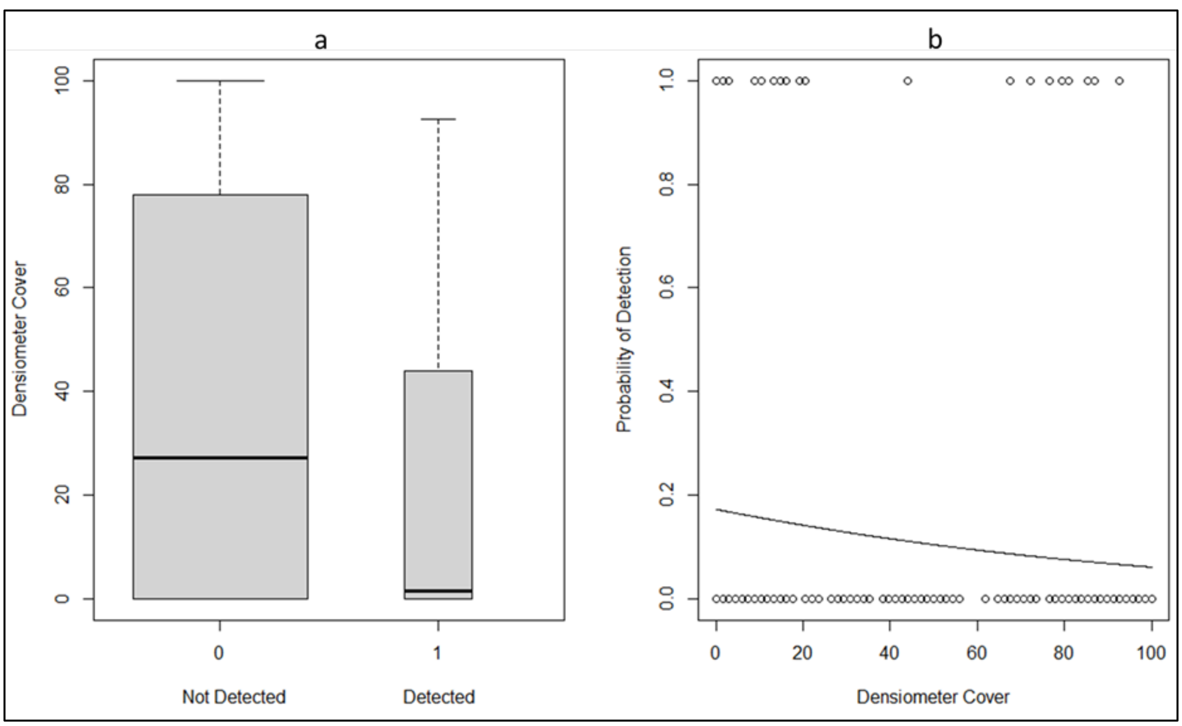


Figure 23 a) Boxplot of densiometer cover during events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected (1) versus not detected (0). Densiometer cover was significantly lower during events where WCT were detected (p = 0.0069). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of WCT by densiometer cover with detection probability curve (p = 0.0218).

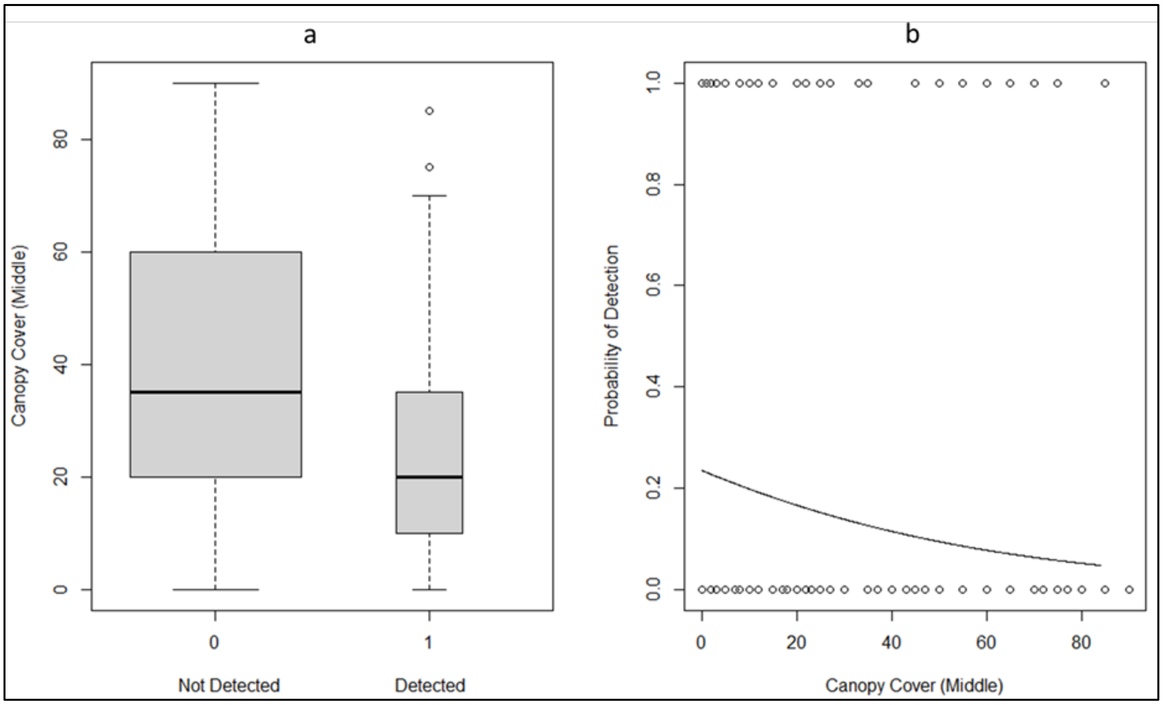


Figure 24 a) Boxplot of canopy cover in the middle height classification (0.5-5 m) during events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected (1) versus not detected (0). Canopy cover was significantly lower during events where WCT were detected ($p = 0.0028$). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of WCT by middle canopy cover with detection probability curve ($p = 0.0054$).

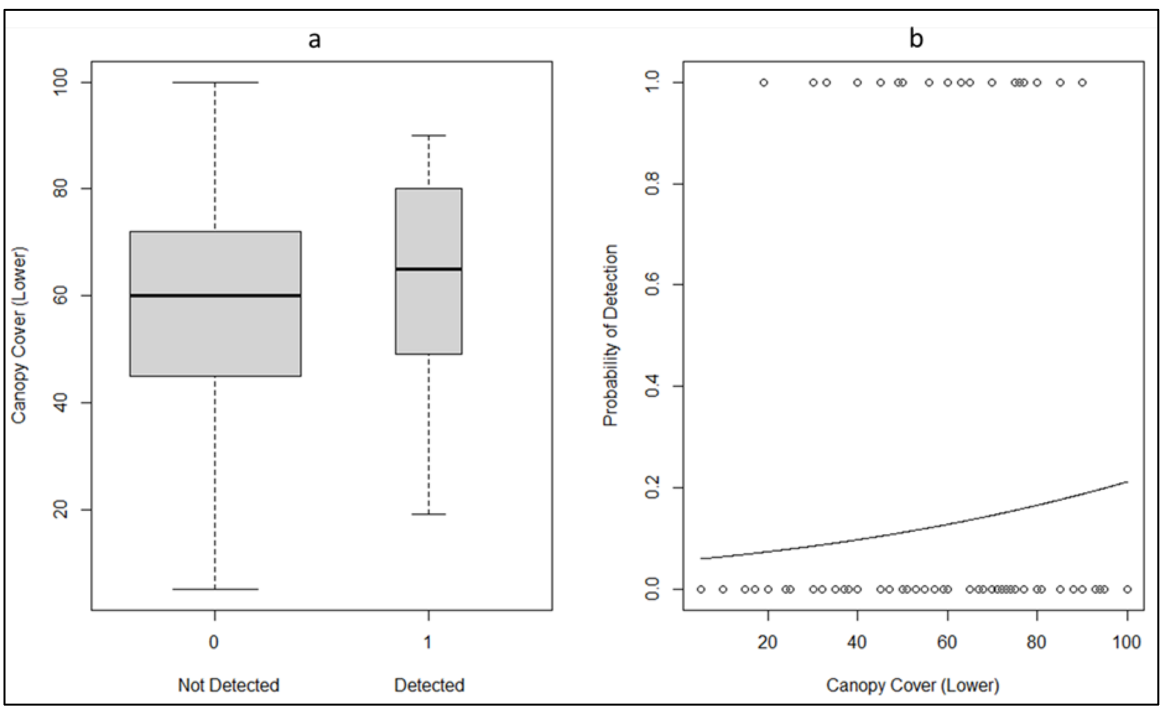


Figure 25 a) Boxplot of canopy cover in the lower height classification (< 0.5 m) during events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected (1) versus not detected (0). Canopy cover was greater during events where WCT were detected, but not at $\alpha = 0.05$ ($p = 0.0823$). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of WCT by lower canopy cover with detection probability curve ($p = 0.0897$).

When we investigated detections of WCT using only the designated NWI classification that the site was bound by, there were four sites (totaling 15 events) that did not overlap with the most current NWI mapper classifications. Pairwise comparison using bound NWI classifications (including "none" for those sites not within an NWI boundary) resulted in the "none" classification having significantly higher detections of WCT compared to all other NWI classifications (p-values ranged from < 0.0001 to 0.0310). Therefore, we used the nearest geomorphologically similar NWI classification for sites falling outside of NWI boundaries in order to elucidate meaningful relationships between the NWI classifications during events where WCT were detected versus those where WCT were not detected.

Detections of WCT differed significantly among the NWI classification that the site was located in ($p < 0.0001$). Pairwise comparison showed that detections of WCT were significantly higher in the Freshwater Emergent NWI classification than Estuarine and Marine ($p = 0.0287$), Freshwater Forested/Shrub ($p = 0.0010$), Lake ($p = 0.0073$), Freshwater Pond ($p = 0.0475$), or Riverine ($p = 0.0205$). Furthermore, detections of WCT were significantly higher in the Freshwater Pond NWI classification than Freshwater Forested/Shrub ($p = 0.0205$) or Lake ($p = 0.0478$). Freshwater Pond (0.571) and Freshwater Emergent (0.357) habitats also made up the highest proportion of events where WCT were detected (Table 17). In addition to NWI wetland classifications, detections of WCT differed significantly among the observed wetland type as determined through on-the-ground conditions at the study site at the time of sampling ($p = 0.0038$). Pairwise comparison indicated that detections of WCT were significantly higher when the observed wetland type at the site was an emergent or a ponded wetland compared to a lake ($p = 0.014$ and 0.037, respectively). Overall, sites with a designated NWI classification or observed wetland type of Freshwater Emergent or Freshwater Pond wetland had the highest potential for and proportion of WCT detections.

Environmental variables were compared among Freshwater Emergent and Freshwater Pond habitats to determine significant correlations between environmental and habitat variables with WCT detection in these wetland types (Table 18). In Freshwater Emergent and Freshwater Pond habitats where WCT were detected, specific conductivity and total cover in the middle canopy later were significantly lower, as with the overall analyses for all wetland types (Table 18). Additionally, pH and Secchi depth were significantly lower during events where WCT were detected ($p = 0.062$ and 0.094, respectively), but not at the $\alpha = 0.05$ level. While there were no further significant correlations found for Freshwater Emergent sites alone, among the Freshwater Pond sites, Secchi depth (m) was significantly lower (e.g., water was more turbid) at sites where WCT were detected ($p = 0.0210$) (Figure 26).

Finally, multiple linear regression was conducted on environmental and habitat variables to determine which variables best explained the likelihood that WCT would be detected at a site. A generalized linear model (GLM) combining middle and lower height canopy covers, NWI classification, observed wetland type at site, Secchi depth, and dominant ground cover type was the best predictor of WCT detection (model coefficients presented in Table 19).

Table 18 Summary of water quality and small-scale habitat variables for all sampling events at Freshwater Emergent and Freshwater Pond wetlands comparing events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected versus those where WCT were not detected. Values are presented as average ± 1 standard error (SE) with range (minimum to maximum) in parentheses. Significant differences based on WCT detections were tested using a Kruskal-Wallis One-Way ANOVA on Ranks. Significance (*p*-values) for each variable provided (α = 0.05) with significant *p*-values italicized.

Variable	WCT Not Detected	WCT Detected	Test Statistic	*p*-value
Water Temperature (°C)	25.75 ± 0.422 (9.9-38.0)	25.63 ± 0.792 (14.5-36.6)	H = 0.184	0.668
Specific Conductivity (µS)	284.99 ± 26.730 (4.8-1681.0)	146.27 ± 16.555 (45.2-449.6)	H = 7.204	0.007
Dissolved Oxygen (mg/L)	6.057 ± 0.3040 (0.24-18.88)	5.242 ± 0.6390 (0.26-13.95)	H = 1.713	0.191
pH	7.451 ± 0.0769 (5.80-10.22)	7.098 ± 0.1390 (5.73-10.13)	H = 3.477	0.062
Secchi (m)[a]	0.497 ± 0.0286 (.01-1.00)	0.378 ± 0.0403 (0.01-1.00)	H = 2.804	0.094
Air Temperature (°C)	26.58 ± 0.437 (6.9-38.5)	26.50 ± 0.696 (14.8-133.9)	H = 0.242	0.623
Densiometer (%)	33.5 ± 3.02 (0-100)	25.0 ± 5.56 (0-93)	H = 2.712	0.100
Total Cover - Upper (%)	13.1 ± 1.81 (0-95)	11.8 ± 3.31 (0-70)	H = 1.055	0.304
Total Cover - Middle (%)	36.4 ± 1.93 (0-90)	27.7 ± 3.64 (0-85)	H = 4.988	0.026
Total Cover - Lower (%)	58.1 ± 1.72 (5-100)	64.3 ± 2.89 (19-90)	H = 2.504	0.114
# of Hydrology Indicators	7.7 ± 0.19 (0-14)	7.2 ± 0.36 (3-14)	H = 2.356	0.125
Total number of events (*n*)	152[b] (range: 141-152)	39	--	--

[a]Secchi maximum detection limit = 1.0 m; recorded as "> 1.000 m" if above detection limit
[b]Some parameters had < 152 events due to localized drought conditions at the site

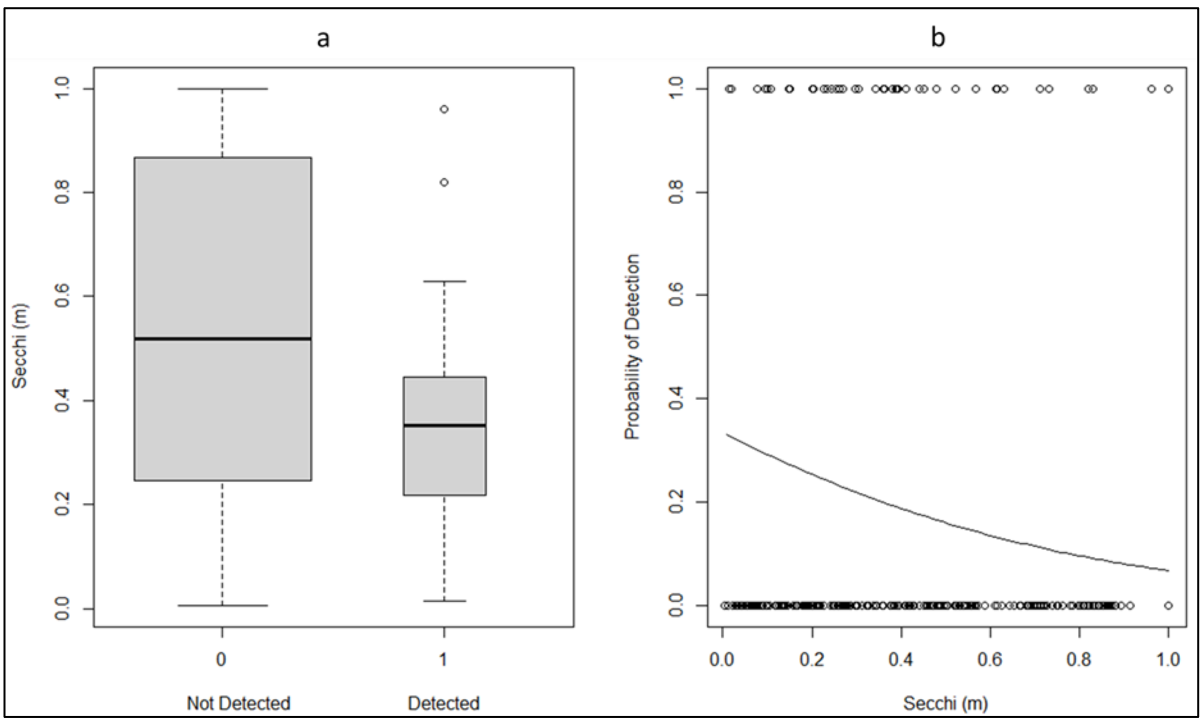


Figure 26 a) Boxplot of Secchi depth (m) for Freshwater Pond wetlands during events where Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) were detected (1) versus not detected (0). Secchi depth was significantly lower during events where WCT were detected (*p* = 0.0210). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of WCT by Secchi depth with detection probability curve (*p* = 0.0155).

Table 19 Model coefficients for best-fit multiple linear regression of environmental and physical habitat variables that explained the small-scale habitat conditions maximizing detection potential of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*). NWI = National Wetland Inventory, SAV = Submerged Aquatic Vegetation, FAV = Floating Aquatic Vegetation.

Variable	Estimate	Standard Error	Z-value	*p*-value
Canopy cover - middle	-0.02843	0.01116	-2.549	*0.0108*
Canopy cover - lower	0.02063	0.01189	1.735	0.0827
NWI classification - freshwater emergent	18.79438	5183.07800	0.004	0.9971
NWI classification - freshwater forested/shrub	-1.24017	5801.91600	0.000	0.9998
NWI classification - freshwater pond	17.62217	5183.07800	0.003	0.9973
NWI classification - lake	2.19418	5792.57200	0.000	0.9997
NWI classification - other	-2.62164	7810.79400	0.000	0.9997
NWI classification - riverine	15.91268	5183.07800	0.003	0.9976
Observed wetland - estuarine	-1.47804	8444.21200	0.000	0.9999
Observed wetland - forest/shrub	1.00798	1.19151	0.846	0.3976
Observed wetland - lake	-18.62934	2190.61900	-0.009	0.9932
Observed wetland - pond	-0.50975	0.46462	-1.097	0.2726
Observed wetland - riverine	-16.34113	3309.43200	-0.005	0.9961
Secchi (m)	-1.54114	0.70727	-2.179	*0.0293*
Dominant ground cover - grasses/herbs	19.59171	3296.28400	0.006	0.9953
Dominant ground cover - open water	17.49269	3296.28400	0.005	0.9958
Dominant ground cover - SAV/FAV	21.21447	3296.28400	0.006	0.9949
Dominant ground cover - trees/shrubs/vines	2.33404	5558.87900	0.000	0.9997

Species Distribution Model Outputs

Overall, 93 total historic occurrences with spatial data were extracted for SDM analyses. Two occurrences from VertNet were lacking dates, so they were excluded as we were unable to determine which temporal model they should be included in. Additionally, 17 historic occurrences occurring between 2001-present day were noted as obscured, so they were excluded from the current SDM. The historic SDM was run with 47 historic occurrence datapoints with corresponding spatial and temporal information (Figure 27). The current SDM was run with 27 historic occurrence datapoints, eight potential detection locations from the current study, and 12 confirmed detection locations from the current study (47 total datapoints) (Figure 28). The detection SDM was run with only the 12 confirmed detection locations from the current study (Figure 29). Omission error rates for all three SDM (Figure 30) had close match between the predicted omission error rate and the true omission error rate during model testing, indicating good model performance. Receiver-operating curves (ROC) were generated for each model. Area under the curve (AUC) averaged from cross-validation for the historic, current, and detection SDMs were 0.796 ± 0.054, 0.820 ± 0.035, and 0.824 ± 0.109, respectively. For all models, AUC was > 0.75, indicating that models fit to the training and test data and did well at predicting the probability of presence from the test data (Stryszowska et al., 2016).

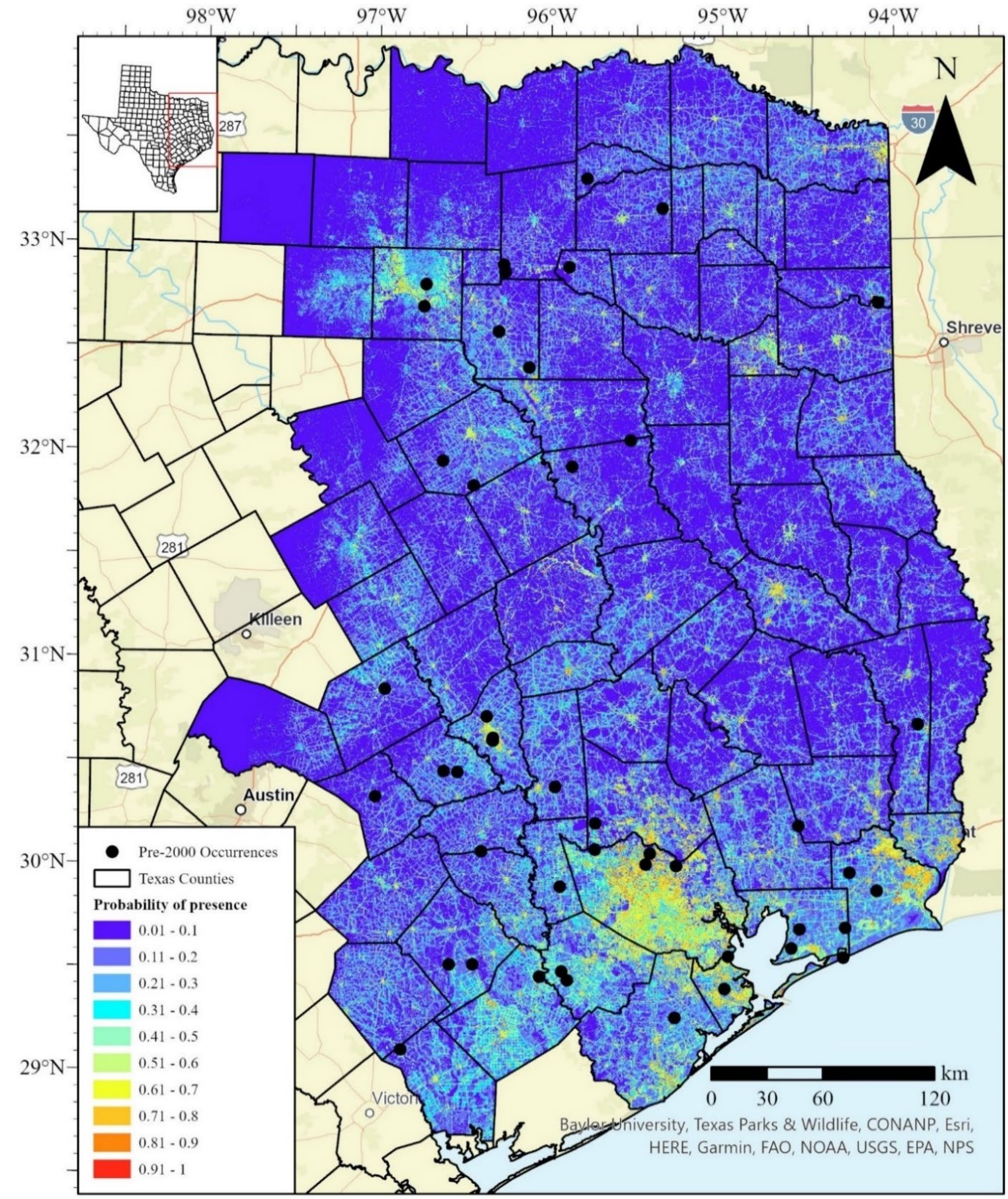


Figure 27 Historic species distribution model (SDM) output from MaxEnt (includes occurrences from 2000 and earlier, *n* = 47) for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) in Texas. Geographic extent is limited to counties within the species historic range + Jackson County. Environmental co-variate input layers included: elevation and slope (USGS, 2013), raw and majority land cover (USGS, 2001), distance between freshwater emergent and ponded wetland types (USFWS, 2022), hydrologic soil group (USDA, 2021), and road density (TXDOT, 2023). Habitat suitability is interpreted from probability of presence with more suitable habitat indicated by warmer colors (red) and less suitable habitat by cooler colors (blue). Black dots represent occurrence locations used in the model.

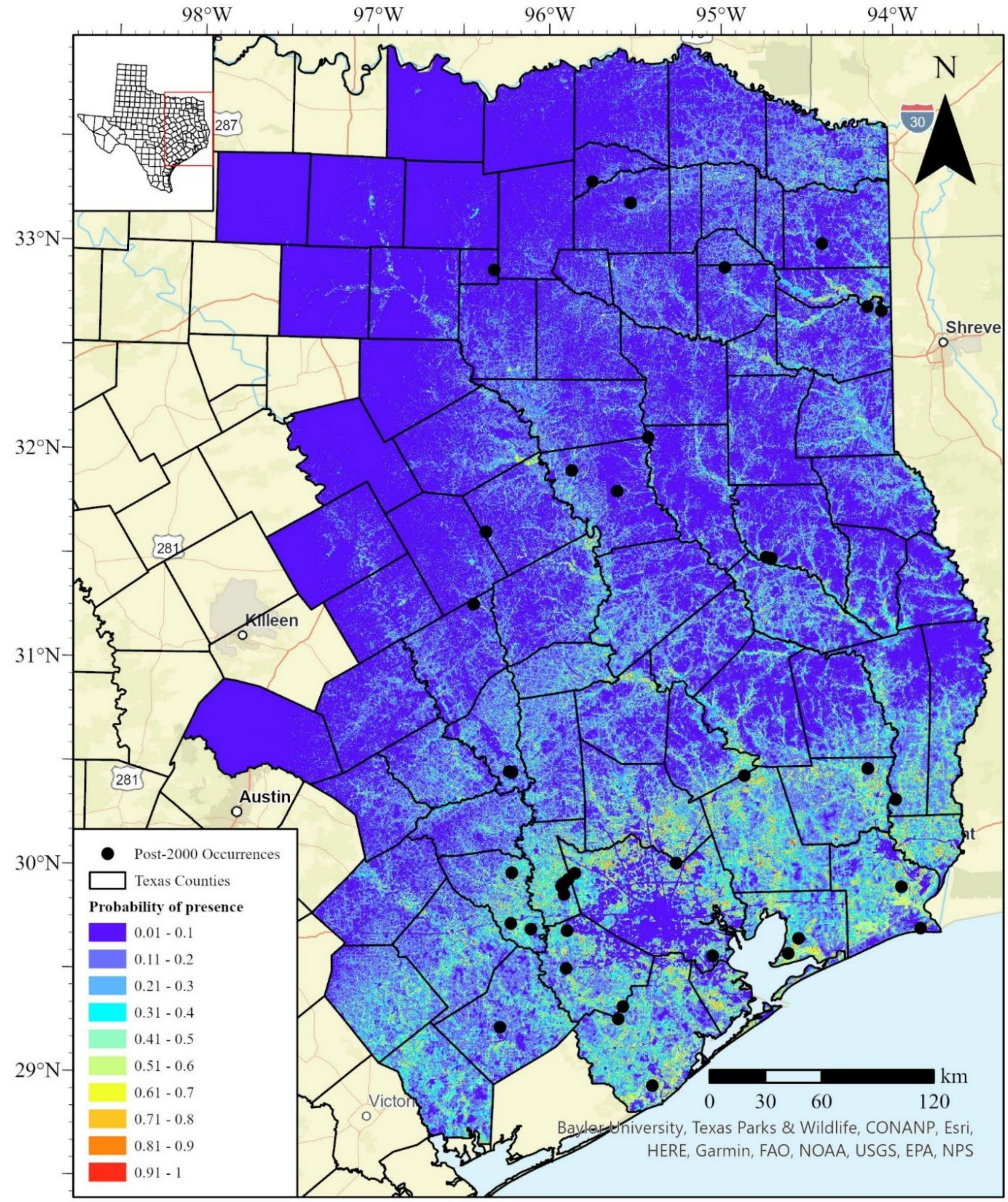


Figure 28 Current species distribution model (SDM) output from MaxEnt including historic occurrences (after 2000, *n* = 27), potential presence localities from the current study (*n* = 8), and confirmed occurrence localities from current study (*n* = 12) for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) in Texas. Geographic extent is limited to counties within the species historic range + Jackson County. Environmental co-variate input layers included: elevation and slope (USGS, 2013), raw and majority land cover (USGS, 2019), distance between freshwater emergent and ponded wetland types (USFWS, 2022), hydrologic soil group (USDA, 2021), and road density (TXDOT, 2023). Habitat suitability is interpreted from probability of presence with more suitable habitat indicated by warmer colors (red) and less suitable habitat by cooler colors (blue). Black dots represent occurrence locations used in the model.

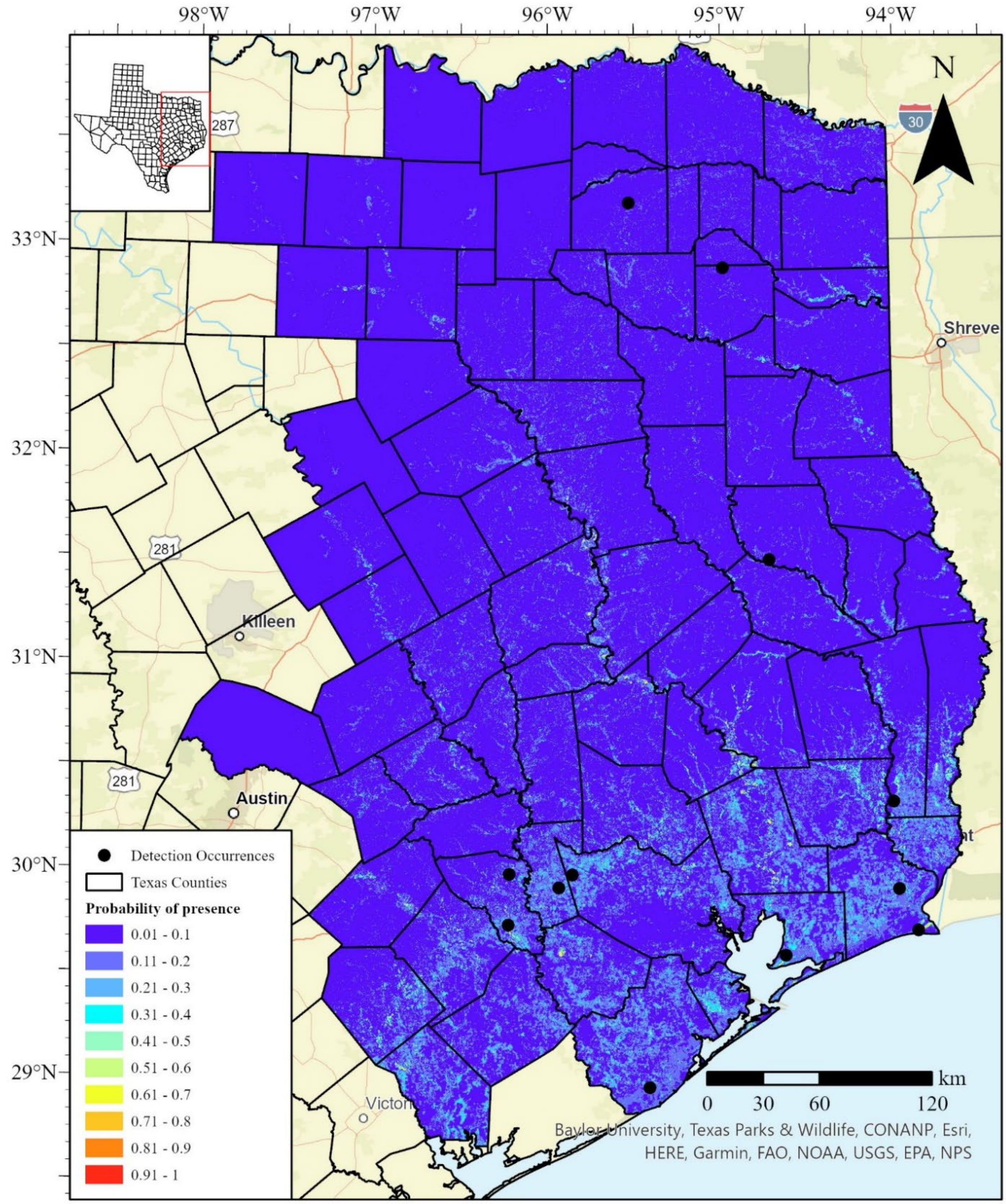


Figure 29 Detection species distribution model (SDM) output from MaxEnt including confirmed detections from this study (n = 12) for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) in Texas. Geographic extent is limited to counties within the species historic range + Jackson County. Environmental co-variate input layers included: elevation and slope (USGS, 2013), raw and majority land cover (USGS, 2019), distance between freshwater emergent and ponded wetland types (USFWS, 2022), hydrologic soil group (USDA, 2021), and road density (TXDOT, 2023). Habitat suitability is interpreted from probability of presence with more suitable habitat indicated by warmer colors (red) and less suitable habitat by cooler colors (blue). Black dots represent occurrence locations used in the model.

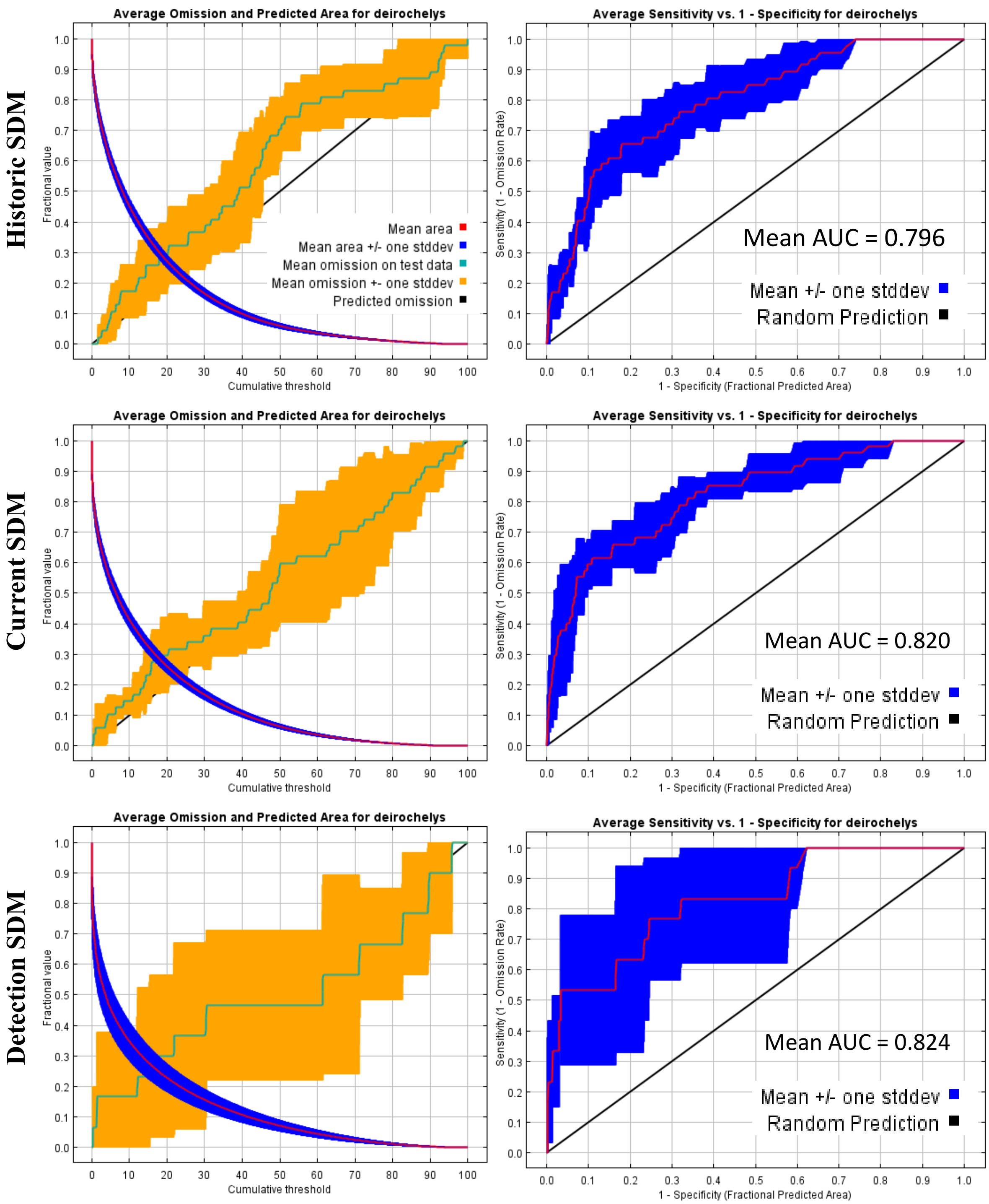


Figure 30 Statistical plots for average omission and predicted area (left column) and model sensitivity and specificity (right column) of historic (top row), current (middle row), and detection (bottom row) species distribution models (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Plots are averaged over five replicates for all models. For omission and predicted area plots, better model performance is indicated by the test omission rate (light blue line in the left column plots) being close to the predicted rate (black line in the left column plots) with yellow areas representative of one standard deviation. For model sensitivity and specificity, better fit is indicated by training of the receiver operating characteristic (ROC) curve (red line in the right column plots) being above the random prediction line (black line in right column plots) and area under the curve (AUC) values > 0.75 with blue areas representative of one standard deviation.

Environmental co-variates for the historic SDM representing over 71% of the total contribution to the model were raw land cover class (47.0%), road density (12.8%), and elevation (11.6%) (Table 20). Environmental co-variates for the current SDM representing over 76% of the total contribution to the model were distance between wetlands (36.1%), elevation (20.7%), and raw land cover class (19.3%). Environmental co-variates for the detection SDM representing over 65% of the total contribution to the model were slope (23.3%), elevation (23.1%), and soil class (19.3%). For the historic, current, and detection SDMs, the most important environmental co-variates were raw land cover class, distance between wetlands, and slope, respectively. Across all models, elevation ranked among the top three co-variates with largest percent contributions and largest permutation importance. With the historic SDM relying heavily on raw land cover class and road density, urban sampling bias was evident in the output (Figure 27). Methods such as targeted species sampling (TGS) have been developed to account for this bias in the occurrence data (Elith et al. 2011; Merow et al. 2013). In the current study, another historic SDM was run in one replicate with raw land cover class and road density excluded as input co-variates (Appendix H). This SDM had relatively good model performance and fit (training AUC = 0.794; test AUC = 0.718) and a more evenly distributed spread of co-variate importance. It resulted in a predicted distribution with some similar spatial patterns to the current and detection SDM, though had more area with higher probability of presence overall and in the northern portion of the study area. However, much of the bias towards roads and cities was absent.

Table 20 Percent contribution and permutation importance of each environmental co-variate averaged over five replicates used in species distribution models (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. Historic SDM used spatial historic occurrence localities for WCT from the year 2000 and earlier (n = 47). Current SDM used spatial historic occurrence localities for WCT occurring after 2000 (n = 27) and potential (n = 8) and confirmed (n = 12) occurrence locations from the current study. Detection SDM used only confirmed occurrence locations from the current study. Top three contributing and importance factors for each model in italics.

	Historic SDM		**Current SDM**		**Detection SDM**	
Environmental co-variate	Contribution	Importance	Contribution	Importance	Contribution	Importance
Distance between wetlands	5.9	2.8	*36.1*	*18.6*	11.9	*18.7*
Elevation	*11.6*	*20.9*	*20.7*	*32.6*	*23.1*	2.6
Land cover class - majority	7.2	*10.3*	7.4	6.0	9.3	10.4
Land cover class - raw	*47.0*	*45.6*	*19.3*	*19.5*	12.1	7.4
Road density	*12.8*	7.0	6.6	15.5	1.1	0.2
Slope	5.8	8.0	3.2	7.1	*23.3*	*48.6*
Soil class	9.7	5.4	6.7	0.8	*19.3*	*12.0*

A jackknife test of co-variate importance was run for each SDM (Figure 31). This test creates multiple versions of the model using simulations of the data to create:1) a test model using all co-variates (analogous to the original MaxEnt output), 2) a series of test models with each co-variate in isolation (dark blue bars in Figure 31), and 3) a series of test models where each co-variate is excluded and the resulting model is created from the remaining variables (light blue bars in Figure 31). For the historic SDM, all jackknife tests showed the model was best informed by raw land cover. For the current SDM, jackknife results of regularized training gain and test gain showed the model was best informed by distance between wetlands, but jackknife of AUC was more variable with elevation causing the largest increase but distance between wetlands caused the largest decrease. For the detection SDM, jackknife of test gain and AUC showed the model was best informed by slope, but jackknife of regularized training gain was more variable with slope causing the largest increase and soil class causing a marginally larger decrease over slope. Overall, at least one environmental co-variate could be identified as the most informational for each model. While the relative importance of the other co-variates differs greatly between the three SDMs, the majority land cover class co-variate typically caused lower gain when in isolation and contributed less than 10% to each model. Six of the nine jackknife tests showed that gain or AUC was higher (light blue bars) than the respective gain from the model using all co-variates (red bars in Figure 31), indicating that predictive performance was improved when this co-variate was excluded. Overall, results indicate that this co-variate was not informative to the model in comparison to other environmental co-variates.

Response curves displaying how each co-variate affected predicted probability of presence for the historic (Figure 32), current (Figure 33), and detection SDM (Figure 34) are shown in order of percent contributions (highest to lowest). The majority land cover class co-variate was not considered due to its low overall importance to each SDM. In the historic SDM, the best estimated probability of detections occurred in raw land cover classes of 24 (e.g., Developed High Intensity), when road density neared 31 km/km^2, at elevations near 5.0 m, in soil classes of 5 (Group A/D), when distance between wetlands was near zero meters, and when topographic slope reached 0.25 degrees. Similarly, the current SDM showed best estimated probability of detection when distance between wetlands and elevation were the same as in the historic SDM, though probability of detection changed with raw land cover class (21; Developed Open Space), soil class (1; Group A), road density (3.5 km/km^2), and slope (0.0 degrees). Response curves for the detection SDM, which used fewer overall occupancy localities, showed similar results to the current SDM, though elevations and road densities near zero and raw land cover class (11; Open Water) were better predictors of WCT presence when compared to the current SDM.

Confidence intervals of the response curves were used to determine the range of environmental conditions that resulted in probability of presence above a certain logistic threshold (i.e., higher habitat suitability found between certain co-variate values). For each SDM, MaxEnt calculated the statistical significance (1-sided *p*-values) of the predicted distribution using various binomial tests of omission, each averaged over the five replicates (Phillips, 2017). Significant tests ($p \leq 0.05$) were evaluated for the most conservative logistic threshold for each SDM. For the historic and current SDMs, the maximum training sensitivity plus specificity threshold resulted in detection probabilities of 0.348 and 0.376, respectively, while the detection SDM threshold resulted in a detection probability of 0.149. The highest detection probabilities (ρ) determined for field protocols within the current study resulted in values near 0.3 (range: 0.287-0.382), therefore, MaxEnt detection probabilities were averaged with the four highest detection probabilities calculated for field protocols and rounded to a final "cutoff" value for easier visual interpretation of the response curve axes. In the historic SDM, the estimated co-variate values above a probability of 0.3 were: raw land cover classes 21-24, 81-82, and 95 for; road densities < 31.0 km/km^2; elevation between 5-175 m; soil classes 1 and 3-7; distance between wetlands ranging < 5500 m; and topographic slope between 0.25-3.25 degrees. In the current SDM, the estimated co-variate values above a probability of 0.3 were: raw land cover classes 11, 21-24, 31, 41-43, 52, 81-82, 90, and 95; road densities < 8.0 km/km^2; elevation between 5.0-125 m; all seven soil classes; distance between wetlands < 500 m; and topographic slope < 2.75 degrees. In the detection SDM, the estimated co-variate values above a probability of 0.3 were: raw land cover classes 11 and 21; elevation < 20 m; soil classes 1 and 7; and no change in slope or distance between wetlands. Range of road density predictor values could not be calculated for the detection SDM.

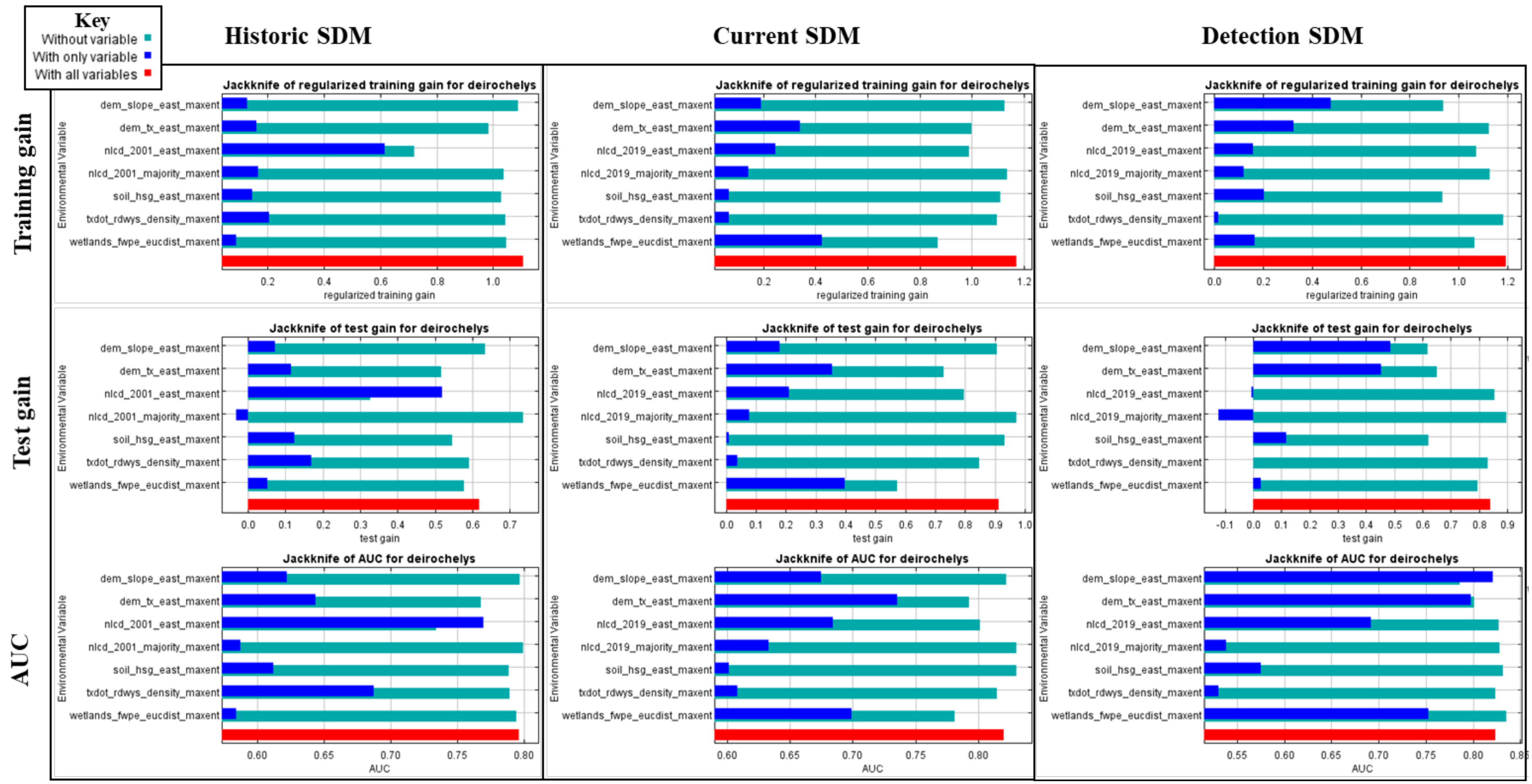


Figure 31 Jackknife test results of regularized training gain (top row), test gain (middle row), and area under the curve (AUC; bottom row) for the historic species distribution model (SDM) (left column), current SDM (middle column), and detection SDM (right column) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Jackknife results show relative importance of environmental co-variates used as inputs with red bars representing models with all co-variates included, dark blue bars representing models with one co-variate used in isolation, and light blue bars representing models with one co-variate omitted. For the historic SDM, all jackknife tests showed the model was best informed by raw land cover. For the current SDM, jackknife results of regularized training gain and test gain showed the model was best informed by distance between wetlands, but jackknife of AUC was more variable with elevation causing the largest increase but distance between wetlands caused the largest decrease. For the detection SDM, jackknife of test gain and AUC showed the model was best informed by slope, but jackknife of regularized training gain was more variable with slope causing the largest increase and soil class causing a marginally larger decrease over slope.

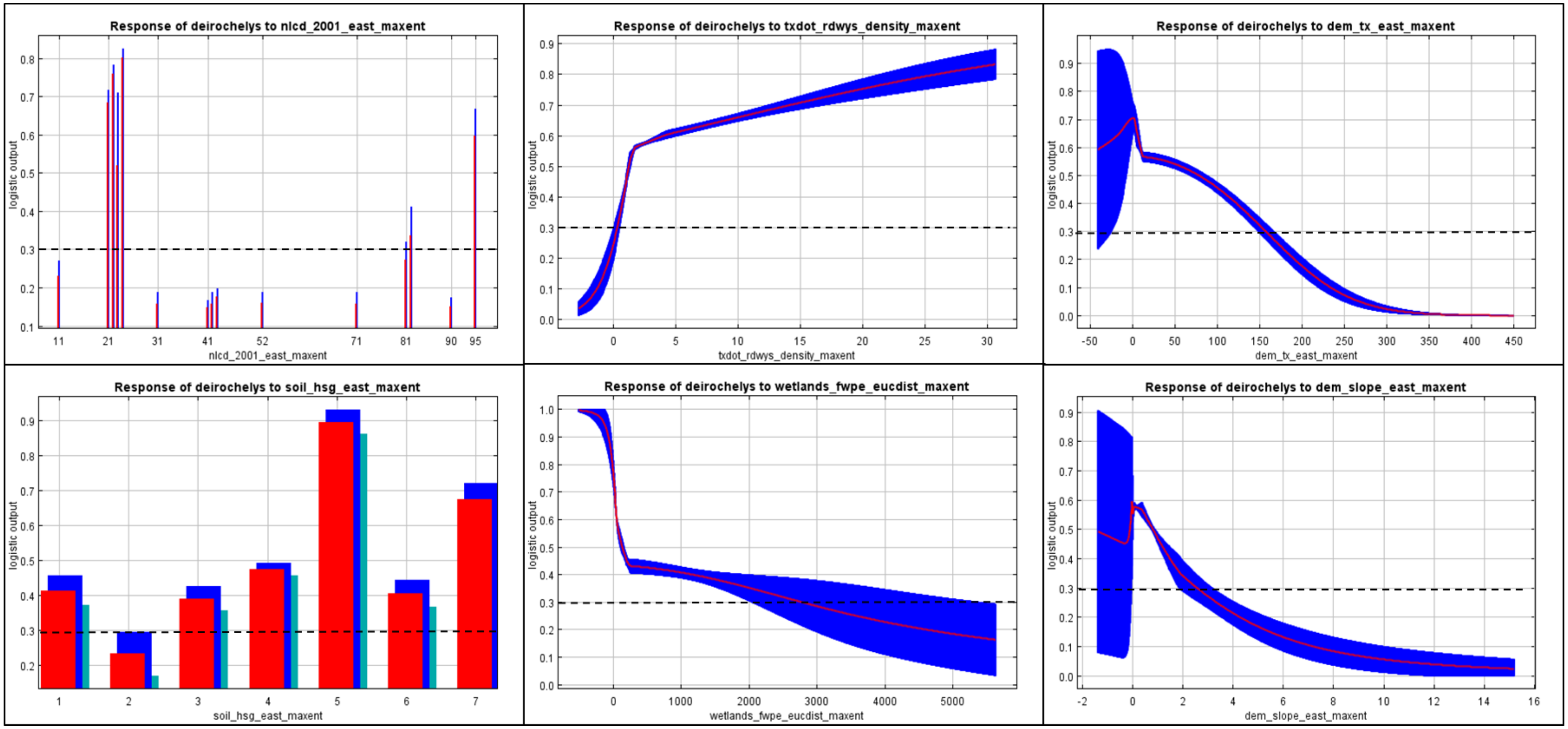


Figure 32 Response curves of six co-variates from the historic species distribution model (historic SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Each curve was generated using only the corresponding co-variate, reflecting dependence of predicted suitability on that co-variate and its possible correlations with other co-variates. Plots show the mean response of the logistic output averaged across the five replicates (red line or bar) with blue areas representing one standard deviation (dark and light blue for categorical co-variates). Plots are ordered based on decreasing percent contribution (left to right, top to bottom). The majority land cover co-variate curve is not shown due to its low overall importance to each SDM. Dashed black lines represent the 0.3 detection probability threshold.

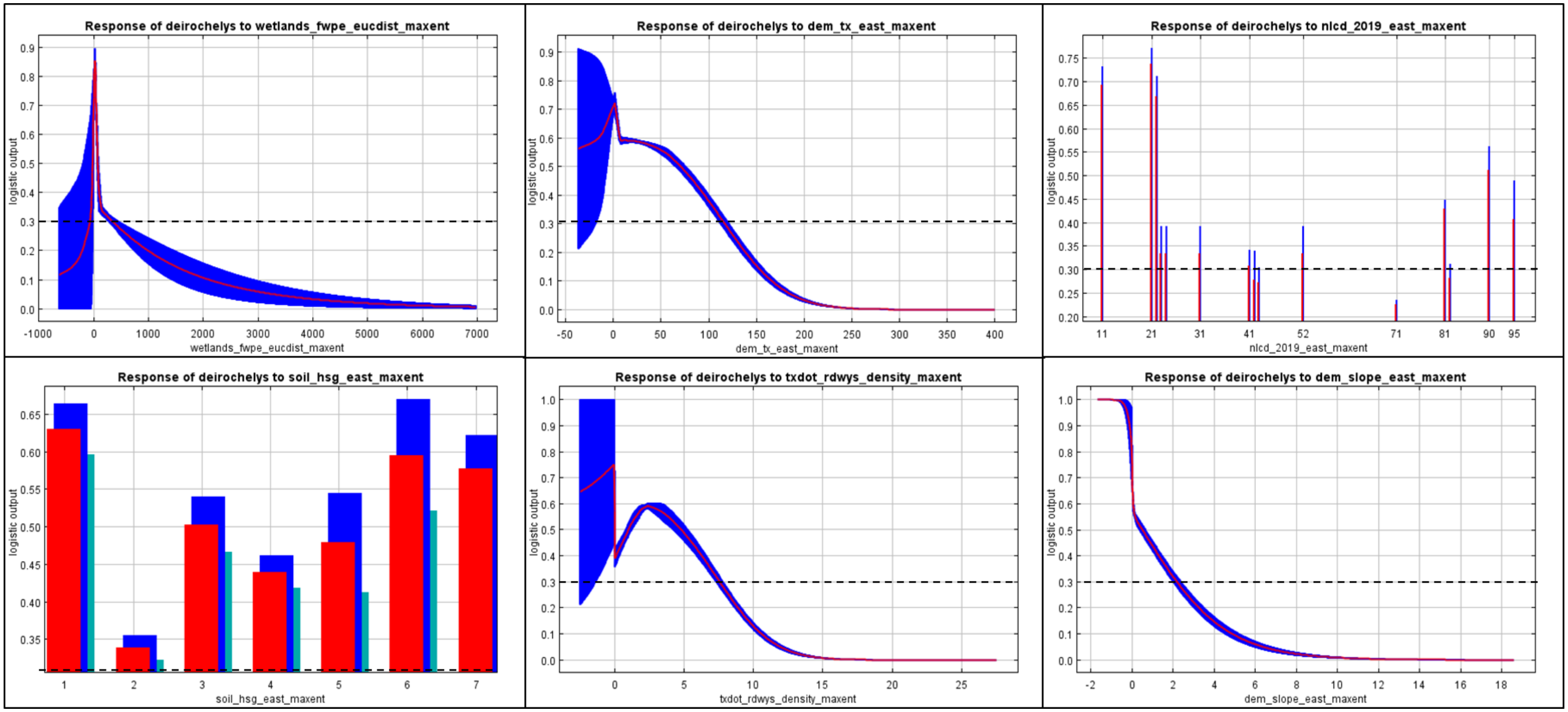


Figure 33 Response curves of six co-variates from the current species distribution model (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Each curve was generated using only the corresponding co-variate, reflecting dependence of predicted suitability on that co-variate and its possible correlations with other co-variates. Plots show the mean response of the logistic output averaged across the five replicates (red line or bar) with blue areas representing one standard deviation (dark and light blue for categorical co-variates). Plots are ordered based on decreasing percent contribution (left to right, top to bottom). The majority land cover co-variate curve is not shown due to its low overall importance to each SDM. Dashed black lines represent the 0.3 detection probability threshold.

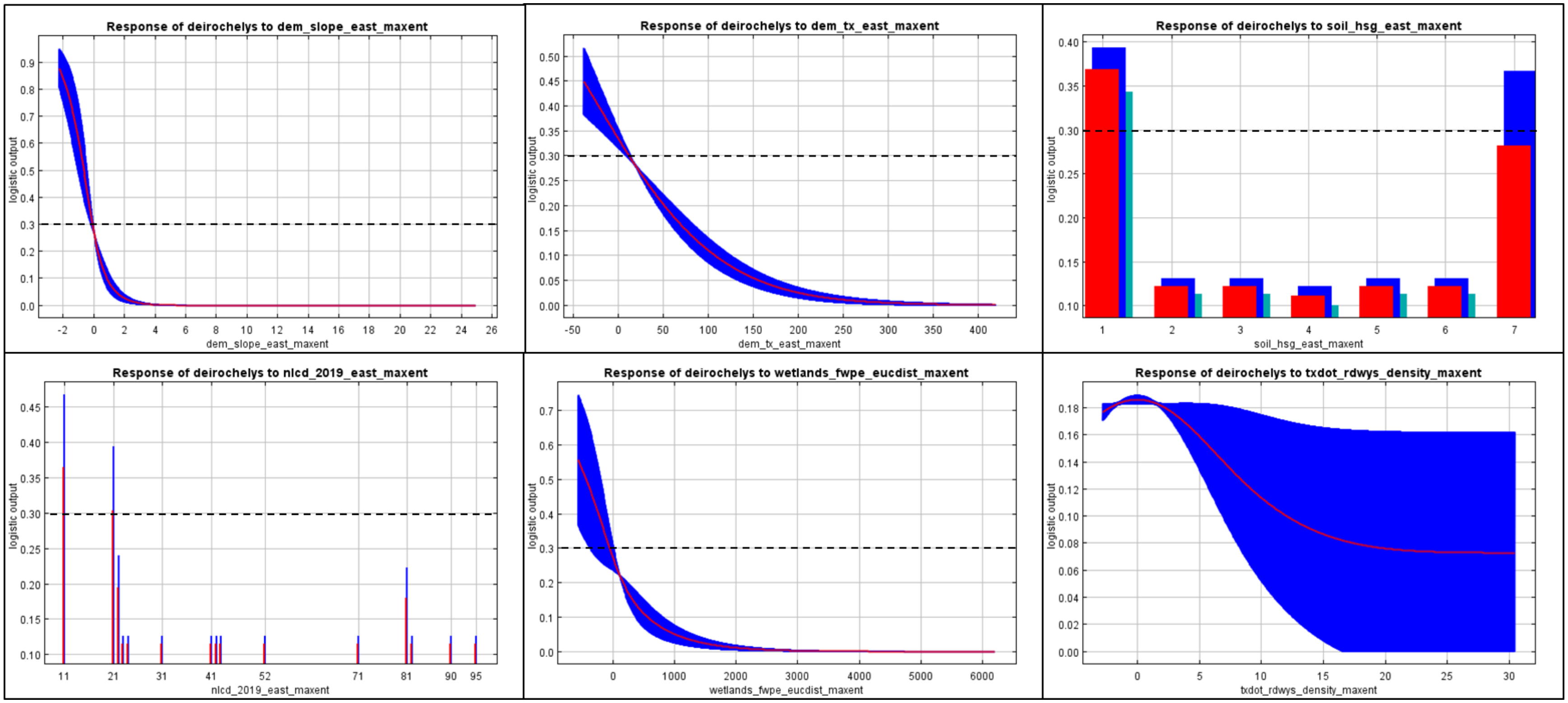


Figure 34 Response curves of six co-variates from the detection species distribution model (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Each curve was generated using only the corresponding co-variate, reflecting dependence of predicted suitability on that co-variate and its possible correlations with other co-variates. Plots show the mean response of the logistic output averaged across the five replicates (red line or bar) with blue areas representing one standard deviation (dark and light blue for categorical co-variates). Plots are ordered based on decreasing percent contribution (left to right, top to bottom). The majority land cover co-variate curve is not shown due to its low overall importance to each SDM. Dashed black lines represent the 0.3 probability threshold.

Protocol Detectability and Comparisons

Across all protocols, 164 detection probability (ρ) models were examined to determine best-fit models for each protocol. A summary of all model iterations can be found in Appendix I. Best-fit models for each protocol resulted in ρ-values ranging from < 0.001 to 0.382 (Table 21). Protocols with the highest probability of detection included camera trap (ρ = 0.382) and resuspended sediment eDNA samples filtered with a 3.0 μm filter (R-3.0; ρ = 0.339). Protocols with the lowest probability of detection included canid scent surveys (CSS; ρ = 0.007) and soil eDNA (ρ = 0.036). Detection probabilities for three protocols could not be calculated due to lack of detections across all sites and events (WS) or inability to assign a detection matrix, even though WCT were detected or reported using those protocols (ORT and RS). For all protocols resulting in detectability co-variates for event (as a function of time) and effort (BAVS, CSS, and $Drone_{M2}$), effort was the better predictor of detection. For all protocols conducted during in-season and out-of-season sampling periods (A-3.0 and R-3.0), detection probability was highest during the in-season sampling period (March-July).

In addition to detection probability (ρ), the proportion of sites occupied according to model estimates (PAO) was also calculated using best-fit models for each protocol. Because models were run using occurrence data for Occupied sites only, if a given protocol was 100% effective at detecting WCT when a site is occupied, PAO can be expected to = 1.00. Of the 11 protocols for which PAO could be calculated, only three resulted in PAO = 1.00 (soil eDNA, A-0.45 eDNA, and hoop trap). Of these three protocols, hoop trap resulted in the highest detection probability (ρ = 0.167).

Table 21 Best fit detectability models for all protocols applied in the current study. Table includes protocol (A-0.45 and R-0.45 = ambient and resuspended eDNA water samples filtered with 0.45 μm filter, respectively; A-3.0 and R-3.0 = ambient and resuspended eDNA water samples filtered with 3.0 μm filter, respectively [“in” and “out” indicate in-season and out-of-season sampling periods]; Soil = soil eDNA samples; BAVS = binocular assisted visual surveys; ORT = online reporting tool; WS = walking survey; RS = road survey; CSS = canid scent survey; Hoop trap = hoop trap survey; Camera trap = game camera trap survey; M2 and P4 = Mavic 2 and Phantom 4 drone platform surveys, respectively), number of sites included in model (*N*), model co-variates for best-fit model, Akaike Information Criteria (AIC), Akaike difference (ΔAIC), Akaike weight (W_{AIC}), proportion of sites occupied according to model estimates (PAO), 95% confidence interval for PAO (95% CI), Pearson's X^2 goodness-of-fit (GoF) test statistic (*t*-value), GoF test significance (*p*-value), and estimated detection probability (ρ). The top three models for each protocol (based on lowest ΔAIC) were tested for GoF. Models resulting in the highest *p*-value were considered “best-fit”. A full list of all model AIC scores can be found in Appendix I.

Protocol	*N*	Model co-variates	AIC	ΔAIC	W_{AIC}	PAO	95% CI	*t*-value	*p*-value	ρ
A-0.45	4	Null	43.56	0.00	0.64	1.00	1.00-1.00	47.0	0.548	0.149
A-3.0 (in)	10	Ψ (wetland)	105.99	0.55	0.28	0.80	0.80-0.80	80.0	0.213	0.287
R-0.45	4	Ψ (wetland)	32.88	0.00	0.56	0.75	0.75-0.75	35.0	0.263	0.114
R-3.0 (in)	10	Ψ (wetland+criteria)	99.34	1.52	0.13	0.60	0.60-0.70	77.1	0.680	0.339
Soil	6	Ψ (habitat)	23.18	2.00	0.23	1.00	1.00-1.00	55.0	0.562	0.036
BAVS	10	ρ (effort) Ψ (habitat)	29.85	0.00	0.42	0.40	0.40-0.40	37.8	0.178	0.117
ORT[a]	--	--	--	--	--		--	--	--	0.020
WS[a]	--	--	--	--	--		--	--	--	0.000
RS[a]	--	--	--	--	--		--	--	--	0.010
CSS[b]	2	ρ (effort) Ψ (habitat+criteria)	15.33	1.30	--	0.50	0.50-0.50	5.8	0.434	0.007
Hoop trap	3	Null	14.81	0.00	0.41	1.00	1.00-1.00	12.0	0.250	0.167
Camera trap	3	Null	14.46	0.00	1.00	0.33	0.33-0.67	16.8	0.674	0.382
M2	4	Ψ (habitat)	20.03	2.38	0.16	0.25	0.25-0.75	20.7	0.103	0.334
P4	4	ρ (event) Ψ (habitat)	23.82	1.32	0.34	0.25	0.25-0.25	3.0	0.685	0.092

[a]Could not calculate detection probability due to matrix incompatibilities.
[b]Unable to compare models for W_{AIC} calculation due to convergence issues between models.

Scores for logistical sub-categories for the protocol comparison rubric were returned from 12 project personnel (technician: $n = 7$; crew lead: $n = 1$; grad student: $n = 2$; co-PI: $n = 2$). Across all logistical sub-categories, scores were provided for 1,064 sub-category and method combinations. Prior to data compilation, 85 (8.0%) scores were removed due to values > 1 around the median for that protocol and sub-category combination. Final scores for all protocols and sub-categories are included in Table 22, with overall ranks for all protocols and sub-categories shown in Table 23.

Final ranks were summed to determine the protocol with the overall lowest cumulative rank value. The protocols with the lowest total ranks (across all sub-categories) were RS (84), BAVS (90), all eDNA sample types (range: 111-131), and WS (114) (Figure 35). Cumulative weight for protocols significantly differed from one another, resulting in three groups ($H = 64.427$, $df = 13$, $p < 0.001$). While there were no significant differences between RS, BAVS, all eDNA protocols, WS, ORT, and hoop trap protocol cumulative ranks, RS and BAVS were ranked significantly lower (e.g., "better") than M2, CSS, camera trap, and P4 protocols (p-values ranged from < 0.001 to 0.011). Additionally, the P4 protocol ranked significantly higher (e.g., "worse") than the ambient (A-3.0), resuspended (R-3.0), WS, and soil eDNA protocols (p-values ranged from 0.025-0.032). No significant differences in cumulative rank were detected between all five eDNA sample protocols or both drone protocols when compared to each other.

Deviation from mean rank for protocols resulting in multiple application types (eDNA and drone) were compared to one another to determine the best recommended application(s) for that protocol type (Figure 36). Comparisons were made with all sub-categories weighted equally. When comparing eDNA protocols ($n = 5$), A-3.0 and R-3.0 sample types had the greatest positive deviation from the mean, though no significant differences were detected in the overall ranks for each protocol ($H = 4.585$, $df = 4$, $p = 0.333$). Conversely, when comparing drone protocols ($n = 2$), the M2 had the greatest positive deviation from the mean and was ranked significantly better than the P4 ($H = 6.833$, $df = 1$, $p = 0.009$).

Protocols were further compared using a series of hypothetical scenarios in order to make best recommendations of protocols to apply in future assessments (Figure 37). In Scenario #1 (all protocols, regardless of detection success, with comparison rubric scores weighted equally), RS and BAVS showed the greatest positive deviation from the mean rank, making them the recommended protocols overall. In Scenario #2 (comparing methods resulting in capture with sub-categories weighted to reflect no concern for overall costs), hoop trap surveys showed the greatest positive deviation from the mean rank, making it the recommended protocol for Scenario #2. In Scenario #3 (comparing methods resulting in detection with sub-categories weighted the same as in Scenario #2), R-3.0 and A-3.0 eDNA protocols showed the greatest positive deviation from the mean rank, making them the recommended protocols for Scenario #3. Finally, in Scenario #4 (comparing all methods from Scenario #3 with sub-category weights adjusted to reflect a concern for cost), a combination of RS, BAVS, A-3.0, and R-3.0 eDNA protocols showed the greatest positive deviation from the mean rank, making them the recommended protocols for Scenario #4.

Table 22 Average scores and results for sub-categories in the protocol comparison rubric.

		Protocol Score													
	Sub-category	**A-0.45**	**A-3.0**	**R-0.45**	**R-3.0**	**Soil**	**BAVS**	**ORT**	**WS**	**RS**	**CSS**	**Hoop Trap**	**Camera Trap**	**M2**	**P4**
LOGISTICS	Permissions	0.6	0.6	0.6	0.6	0.6	0.5	0.9	0.5	0.5	3.0	1.6	1.3	3.0	3.0
	Planning	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.7	0.7	1.9	1.7	1.6	2.4	2.4
	Difficulty of gear transport	1.1	1.1	1.1	1.1	0.9	0.8	0.8	0.8	0.7	1.3	1.8	1.7	2.0	2.0
	Difficulty of implementation	0.5	0.5	0.5	0.5	0.5	0.6	0.8	1.3	0.4	2.3	1.2	1.1	1.0	1.0
	Time and maintenance	1.5	1.5	1.5	1.5	0.4	0.4	0.8	0.4	0.5	0.8	1.8	1.8	2.5	2.5
	Technical expertise	1.2	1.2	1.2	1.2	0.6	0.8	0.8	0.8	0.7	2.1	1.4	1.4	2.6	2.6
	Performance variability	0.6	0.6	0.6	0.6	0.6	1.2	1.1	0.9	1.2	1.0	0.5	1.1	1.7	1.9
	Potential for failure	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.5	0.7	1.0	1.0	1.5	1.5	1.5
	Resolution	0.8	0.8	0.8	0.8	0.9	0.9	1.4	0.6	0.8	0.9	0.6	1.0	0.6	0.6
STATISTICS	Number of personnel (N_{pers})	8	8	8	8	7	4	2	4	4	5	6	7	7	7
	Number of sites (N_{sites})	4	4	4	4	6	4	2	3	6	1	1	2	2	2
	Detection probability (ρ)	14.9%	28.7%	11.4%	33.9%	3.6%	11.7%	2.0%	0.0%	1.0%	0.7%	16.7%	38.2%	33.4%	9.2%
	"Catch" per unit effort (CPUE)	0.5833	0.9739	0.4800	0.9825	0.1412	0.0896	0.0204	0.0000	0.0570	0.0531	0.0018	0.0006	0.6435	0.1176
	Detection Proportion (Det%)	14.6%	24.3%	12.0%	24.6%	2.4%	10.2%	20.4%	0.0%	3.8%	10.5%	18.2%	0.0%	13.0%	5.9%
	Geographic coverage (G_{cov})	0.0002%	0.0002%	0.0002%	0.0002%	0.0000%	1.94%	100.0%	5.24%	0.0057%	92.15%	0.0174%	0.0169%	31.11%	30.75%
	Stages of analysis (N_{stages})	10	10	10	10	10	5	5	5	5	5	5	7	7	7
COSTS	Start-up costs (C_{start})	$2,500	$2,500	$2,500	$2,500	$1,550	$500	$550	$553	$553	$5,423	$833	$653	$3,889	$6,713
	Cost per event (C_{event})	$896	$888	$1,126	$1,115	$529	$290	$2,945	$345	$250	$1,461	$1,243	$1,796	$1,685	$1,685
	Time (pre-field) (T_{pre})	0.25	0.25	0.25	0.25	0.25	0.25	10.00	0.25	0.25	0.50	0.50	0.25	1.00	1.00
	Time (field) (T_f)	0.25	0.25	0.25	0.25	0.17	0.25	1.00	0.33	0.17	1.00	1.00	0.50	0.50	0.50
	Time (post-field) (T_{post})	0.38	0.37	0.62	0.61	0.16	0.09	1.25	0.13	0.09	0.09	0.22	1.38	0.50	0.50

Table 23 Final ranks for all protocols and sub-categories used in the protocol comparison rubric.

		Protocol Rank													
	Sub-category	**A-0.45**	**A-3.0**	**R-0.45**	**R-3.0**	**Soil**	**BAVS**	**ORT**	**WS**	**RS**	**CSS**	**Hoop Trap**	**Camera Trap**	**M2**	**P4**
LOGISTICS	Permissions	4	4	4	4	4	2	9	1	3	12	11	10	12	12
	Planning	6	6	6	6	4	1	4	2	3	12	11	10	13	13
	Difficulty of gear transport	6	6	6	6	5	3	2	3	1	10	12	11	13	13
	Difficulty of implementation	3	3	3	3	2	7	8	13	1	14	12	11	9	9
	Time and maintenance	7	7	7	7	1	2	5	2	4	5	11	12	13	13
	Technical expertise	6	6	6	6	1	4	3	4	2	12	11	10	13	13
	Performance variability	2	2	2	2	6	12	10	7	11	8	1	9	13	14
	Potential for failure	5	5	5	5	4	3	5	1	2	11	10	12	12	12
	Resolution	6	6	6	6	10	12	14	4	5	11	1	13	2	2
STATISTICS	Number of personnel (N_{pers})	11	11	11	11	7	2	1	2	2	5	6	7	7	7
	Number of sites (N_{sites})	3	3	3	3	1	3	12	8	1	13	13	9	9	9
	Detection probability (ρ)	6	4	8	2	10	7	11	14	12	13	5	1	3	9
	"Catch" per unit effort (CPUE)	4	2	5	1	6	8	11	14	9	10	12	13	3	7
	Detection Proportion (Det%)	5	2	7	1	12	9	3	14	11	8	4	13	6	10
	Geographic coverage (G_{cov})	10	10	10	10	14	6	1	5	9	2	7	8	3	4
	Stages of analysis (N_{stages})	10	10	10	10	10	1	1	1	1	1	1	7	7	7
COSTS	Start-up costs (C_{start})	8	8	8	8	7	1	2	3	3	13	6	5	12	14
	Cost per event (C_{event})	6	5	8	7	4	2	14	3	1	10	9	13	11	11
	Time (pre-field) (T_{pre})	1	1	1	1	1	1	14	1	1	10	10	1	12	12
	Time (field) (T_f)	3	3	3	3	1	3	12	8	1	12	12	9	9	9
	Time (post-field) (T_{post})	8	7	12	11	5	1	13	4	1	1	6	14	9	9

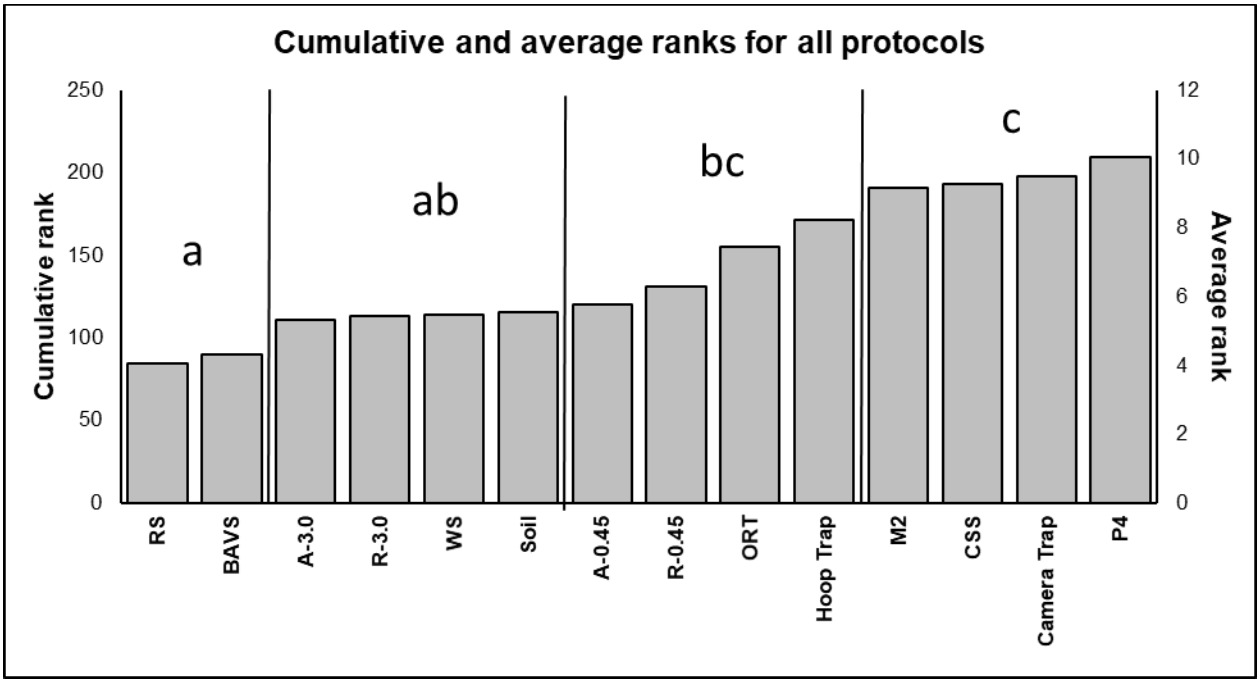


Figure 35 Cumulative and average ranks for all protocols used in surveys of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas. Lower cumulative or average rank represents protocols that were considered "best" across all comparison rubric categories and sub-categories. Protocols were split into three statistical groupings (H = 64.427, df = 13, p < 0.001; groups indicated by letters above bars). There were no significant differences in cumulative or average rank between all eDNA or all drone protocols when compared to one another.

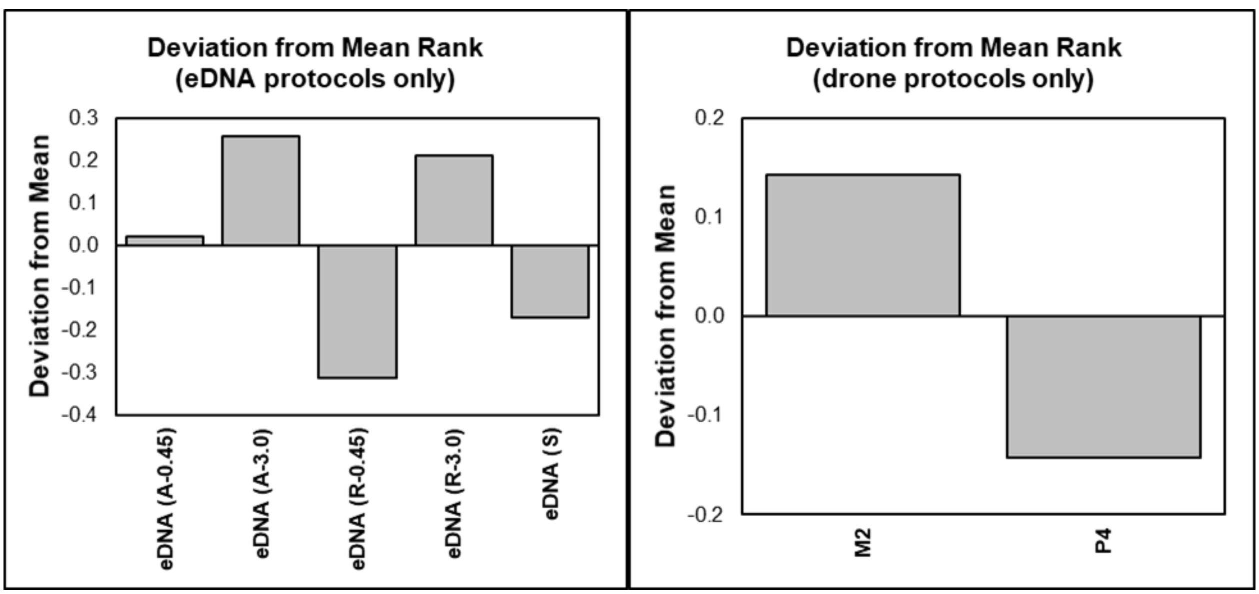


Figure 36 Results of comparison for protocols with multiple application types using deviation from mean rank with all protocol comparison rubric sub-categories equally weighted. Left: Environmental DNA (eDNA) protocols compared to one another with ambient water and resuspended sediment samples filtered using 3.0 μm filters (A-3.0 and R-3.0, respectively) showing the greatest positive deviation from the mean. Right: Drone protocols compared to one another with the Mavic 2 Dual platform (M2) showing a greater positive deviation from the mean than the Phantom 4 Multispectral platform (P4).

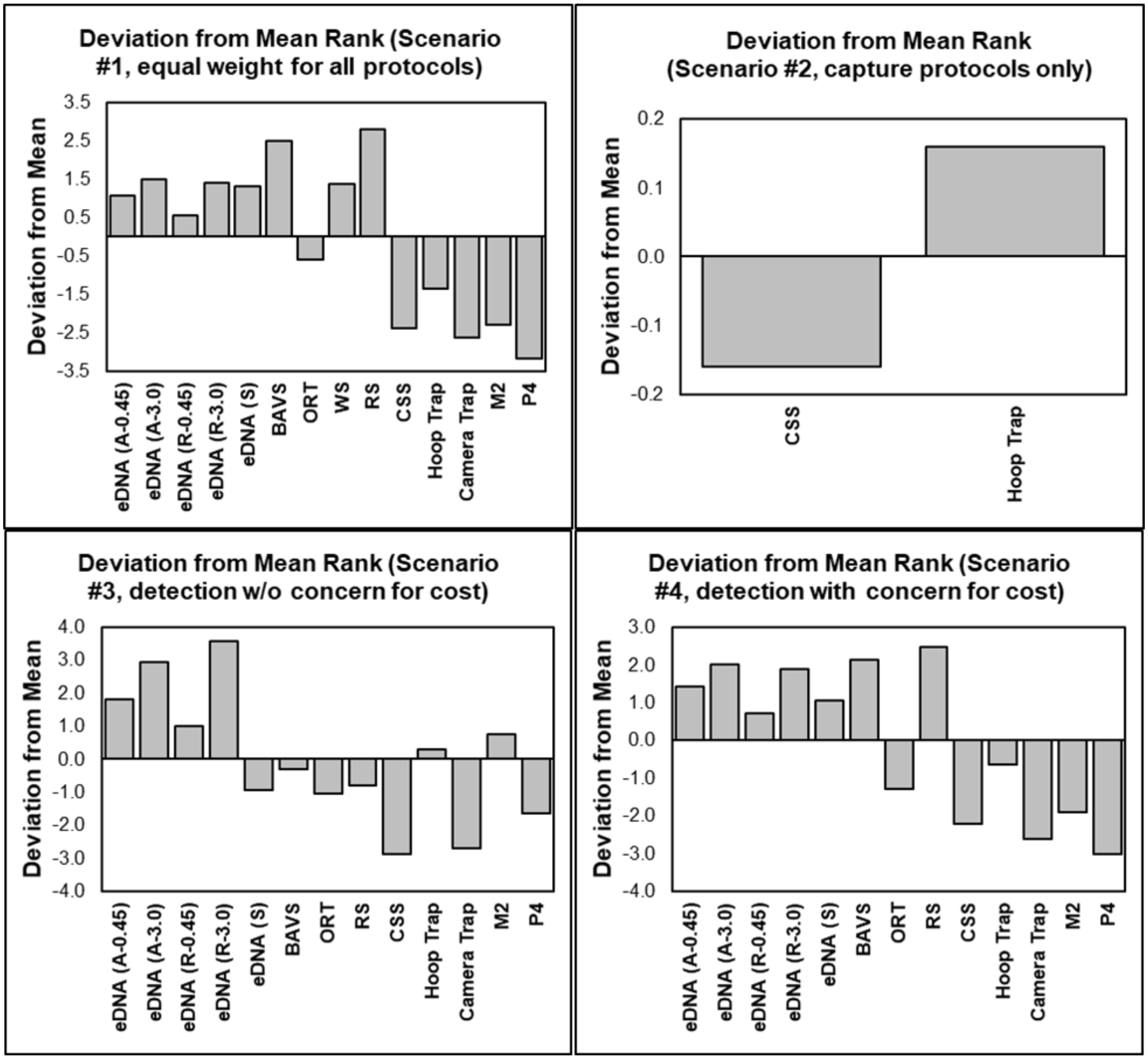


Figure 37 Results of scenarios applied to the protocol comparison rubric for detection of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*). Scenario #1 (top left) represents a "null" test and includes all protocols from the current study and equally weighted sub-categories showing a combination of road surveys (RS) and binocular assisted visual surveys (BAVS) as best-recommended protocols for Scenario #1. Scenario #2 (top right) includes protocols resulting in WCT captures (CSS = canid scent surveys and Hoop Trap = trapping surveys) with sub-categories weighted to represent "no concern for costs". Hoop trap surveys are the best-recommended protocol for Scenario #2. Scenario #3 (bottom left) includes all protocols resulting in WCT detections and uses the same sub-category weights as Scenario #2. A combination of resuspended and ambient environmental DNA samples filtered with a 3.0 µm filter (R-3.0 and A-3.0, respectively) are the best-recommended protocols for Scenario #3. Scenario #4 (bottom right) includes all protocols from Scenario #3 with sub-categories weighted to reflect "concern for cost". A combination of RS, BAVS, R-3.0, and A-3.0 protocols are the recommended protocols for Scenario #4.

DISCUSSION

Current Range and Distribution of Western Chicken Turtles in Texas

We conducted field surveys in 33 (41.8%) of the 79 counties that comprise the WCT historic range in Texas. In addition to field survey efforts, we received a photo-verified report of a WCT in one other county. Of the 34 counties represented in the current study, we had confirmed detections in 10 and potential detections in an additional three. Additionally, historic occurrence locations and locations resulting from personal communications that were used in randomized site selection and SDM resulted in records from an additional 11 counties (iNaturalist, 2020; VertNet, 2020; GBIF, 2022). Recent studies have confirmed presence of WCT in five counties (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019; Bowers et al., 2021, 2022a). Across all data compiled, WCT reports from 24 (30.4%) of the 79 counties within the historic range resulted in detections (confirmed or potential) or historic occurrences. These counties include: Anderson, Austin, Brazoria, Brazos, Cass, Chambers, Delta, Falls, Fort Bend, Freestone, Hardin, Harris, Harrison, Hopkins, Jasper, Jefferson, Liberty, Milam, Nacogdoches, Robertson, Rockwall, Upshur, Waller, and Wharton (a full list of counties within the WCT historic range with updated occupancy status can be found in Appendix G). While we did not sample any locations within Falls county, we did sample locations in Fort Bend, Hardin, Harrison, and Milam counties, but did not return any positive detections of WCT. Though we are able to provide short-term distribution and habitat association trends in approximately half of the known historic range for the species, we recommend continued efforts in areas not included as part of the current survey in order to compile a more holistic view of the current range and distribution of WCT in Texas.

Overall, WCT were detected during all months of the in-season sampling period (March-July), though not always at sites that were considered occupied or during every event. Additionally, WCT were detected during out-of-season sampling efforts and through photo-verified reports to the online reporting tool in all months except February and November. Previous studies of WCT have divided activity patterns into two primary seasons: aquatic activity season and aestivation season (Bowers et al., 2021, 2022a). During the aestivation season, telemetry and trapping efforts suggest that no WCT remained in aquatic habitats during the late summer or fall (Dinkelacker and Hilizinger, 2009; McKnight et al., 2015; Bowers et al., 2021, 2022a). While we focused the majority of our field efforts on the in-season (e.g., aquatic activity season) sampling period, we were able to detect WCT using eDNA, BAVS, and photo-verified reports through our online reporting tool during the out-of-season (e.g., aestivation season) period as well. We recommend further evaluation of WCT activity, movement patterns, and habitat use during the out-of-season sampling period in order to better elucidate annual requirements of the species for future conservation efforts.

Habitat Associations of Western Chicken Turtles in Texas

Small-scale habitat associations

During year 1, we were unable to sample as large a spatial area as originally intended due to the travel restrictions put in place because of the COVID-19 pandemic. That same year, many of the sites that were sampled faced prolonged drought conditions, which may have ultimately affected our ability to detect WCT at that time. In year 2, we were able to expand spatial distribution of survey locations, but many areas faced record-breaking precipitation (and flood) conditions, especially in the earlier part of the season. Overall, restrictions and major climatic events across multiple field seasons caused for inevitable variability in our data, so the following analyses were conducted on a per event basis and only for the in-season sampling period.

Based on our results, detection of WCT is most likely in Freshwater Emergent wetlands, with Freshwater Ponds the second and only other wetland classification for statistically increased detection likelihood. All other major wetland classifications were surveyed, but only one classification had positive detections (Riverine = 3 events with detections). This survey location was comprised of a disconnected depression within the floodplain of a river and surrounded by cattle pasture. A dam was installed at this location > 20 years ago in order to hold water for cattle, but the current NWI classification for this location does not represent its current function as an anthropogenically ponded habitat. We believe the NWI classification in this instance is outdated as the area is not functioning as riverine wetland habitat according to the NWI definition requiring that the wetland be contained within a channel in order to be classified as "Riverine" (Federal Geographic Data Committee, 2013). Additionally, there were two other locations (totaling 15 events with detections) that did not fall within the boundaries of an NWI category. Both sites were determined to be functioning as Freshwater Emergent wetlands based on field observations and proximity to the next geomorphologically similar wetland classification in the NWI. While the NWI classification system is a valuable tool for large-scale wetland habitat mapping, we caution use of this and other landscape scale datasets in analyses of potential small-scale habitat associations. In many cases, landscape scale data, such as the NWI, are not designed for smaller-scale regional or state-level assessments because maps are produced at resolutions resulting in large spatial gaps or generalizations for large spatial areas. For the purpose of this study, the NWI boundaries were key in the randomized site design, but considerations and alternative plans were made in the planning phase of the study due to existing knowledge of the NWI dataset and limitations or data gaps occurring at smaller-scale resolutions. Data from the NWI, and other landscape scale datasets, should be used with caution when extrapolating potential conservation strategies for WCT at the small-scale habitat level, as we have shown that WCT may be observed, detected, or found in areas not contained or associated with landscape scale datasets, like the NWI.

Similar to analyses based on NWI classification, emergent and ponded functional wetland types (as observed by the field team at the time of each sampling event) also had the highest likelihood of WCT detections. Due to the dynamic nature of ephemeral wetlands, the observed wetland type varied regularly among visits to the same location. For example, in two events with WCT detections (at different sites), the field team assigned the observed wetland type as Forest/Shrub, though overall, these wetlands were classified by the NWI as Freshwater Emergent and Freshwater Pond. Both events occurred in June of 2020, during prolonged drought conditions and at times when denser, larger, and more perennial vegetation were able to encroach on the wetland area. We caution resource managers to also consider variability in habitat type based on prolonged climatic events (drought, flood, etc.) as our results suggest that variation within small-scale habitat areas can change year-over-year.

As corroborated by densiometer and canopy cover analysis, wetlands with minimal canopy cover (e.g., not Forest/Shrub) but higher ground cover (e.g. Freshwater Emergent) provide the highest likelihood of detecting WCT. Habitats with events resulting in decreased densiometer and mid-level canopy cover had higher likelihood for detecting WCT. In other words, while WCT appear to prefer a lack of canopy cover, they do prefer a higher level of ground-level cover, specifically cover provided by in-water (as floating or submerged aquatic vegetation) and emergent vegetative species. These conditions are also indicative of Freshwater Emergent and Pond wetland types, further supporting small-scale habitat analyses for these NWI and observed wetland classifications.

Within Freshwater Pond wetland classification areas, we found that WCT detections were significantly correlated with reduced water clarity. Increased water turbidity is common in dynamic ephemeral emergent and ponded wetlands. In wetland areas (including ponds) with shallower overall water depths and variable water levels, there is increased potential for mixing and resuspension of fine sediments within these systems ultimately leading to reduced water clarity. During events were WCT were detected, the highest proportion of detections were made in locations where the dominant substrate type on the shoreline and in-water was clay. While some studies have evaluated importance of sandier substrates (Buhlmann, 2009; Ryberg et al., 2016), presence of clay within these dynamic freshwater emergent and ponded wetlands may also be a key component to the WCT habitat selection. In the context of our study, increased Secchi depth may not be a direct indicator of WCT presence or absence, but more an overall important habitat criterion to consider as it has major implications for the type and quality of available habitat in a given area.

While it is understood that WCT are a freshwater aquatic turtle species, our randomized site selection design resulted in a handful of brackish and marine wetland habitats being sampled. These data allowed for the development of a prediction of detection curve across the wide range of specific conductivities observed at our study sites (ranging from < 5 to > 22,000 µS). No detections occurred at sites with even slightly elevated specific conductivity (> 500 µS) and do not expect WCT to be detected (or present) at sites with conductivities greater than 1,500 µS.

In year 2, our ORT resulted in reports received early enough for locations to be included in field efforts during years 2 and 3. In three instances, we received photo-verified reports of WCT which resulted in sites being added to the survey design. At all three locations, field efforts across multiple protocols resulted in no confirmed detections of WCT. At one of these locations, WCT were potentially detected by eDNA protocols (three occurrences with ambient water samples and two occurrences with resuspended sediment samples both filtered on 3.0 µm filters; A-3.0 and R-3.0, respectively), but at no time were WCT detected using BAVS, drone, or CSS (even after CSS intensifications in year 3). Though no confirmed detections of WCT were made at sites resulting from ORT reports, we were able to use the information in order to update overall occupancy status for the county in which these locations reside. This type of "Local Ecological Knowledge" reporting has been shown to be integral in filling knowledge gaps which may arise from broad scale wildlife surveys (Anadon et al., 2009; Crocetta et al., 2017; Gordon et al., 2023). In all three reports to the ORT, the WCT was observed traveling through the habitat (across a lawn or roadway). This suggests that WCT can and do travel between preferred wetland habitats, further supporting the theory of larger-than-anticipated movement patterns, or even variations of nomadism in the species (Bowers et al., 2021). This also indicates that non-wetland habitats can act as critical corridors supporting WCT populations. This aspect of habitat connectivity may be important due to the WCT unique life history of utilizing ephemeral wetland habitats that are in constant flux. Additionally, recent surveys of WCT in southeast Texas showed that individuals utilized between one and six wetlands within a mosaic environment (Bowers et al., 2021). Connection of spatially isolated preferred habitats may be a key conservation strategy for the species, allowing for continued (or increased) potential for mating and gene mixing.

While the use of Freshwater Emergent and Freshwater Pond wetland types by WCT in Texas has been previously reported (Bowers et al., 2021, 2022a), to the best of our knowledge, no studies have employed a randomized survey design that specifically encompassed other wetland types. We are confident after the substantial effort included in this study in all wetland categories that future work can and should focus efforts on Freshwater Emergent and shallow (typically vegetated) Freshwater Pond wetland types to maximize WCT detections. Specifically, the small-scale habitat variables that are most informative for WCT detectability are middle (0.5 – 5 m) and lower (< 0.5 m) height canopy covers, NWI category (or nearest geomorphologically similar NWI category) of a given site, observed wetland type at a given site, Secchi depth, and the dominant ground cover type (especially inclusive of floating or submerged aquatic vegetation). Additionally, it should be understood that WCT can and do travel among preferred wetland habitats, and non-wetland corridors may be an important factor to consider in conservation strategies for this species.

Landscape scale habitat associations

In general, SDM indicate the estimated probability of presence (warmer, e.g., red cells) or absence (cooler, e.g., blue cells) on a binomial scale (0-1) across each cell of the study area based on input from co-variates. The statistical measures produced by MaxEnt indicated that each of our SDM had good performance, fit, and predictive power (AUC > 0.75 for all models; Stryszowska et al., 2016). The resulting distribution maps are a transformation of the raw MaxEnt relative occurrence rate which is defined as "the probability that a cell is contained in a collection of presence samples as a function of the environmental co-variates" (Merow et al., 2013). However, interpreting these distribution maps as true probability of presence may not be entirely accurate, due to the strong assumptions MaxEnt makes about sample effort and species prevalence in the landscape (Elith et al., 2011; Merow et al., 2013). Instead, the SDMs from this study should be interpreted as the relative suitability of habitat for WCT within the study area.

In the historic SDM, raw land cover and road density co-variates contained the most useful information for the model. These co-variates were representative of anthropogenic influences on WCT and this model iteration often predicted higher habitat suitability in or around highly urbanized areas (e.g., Houston, Dallas, and Beaumont) and roadways. These predictions are likely due to inherent bias in the historic occurrence data and not truly indicative of suitable habitat across the WCT historic range. Often, historic species records are reported in areas that are most easily accessible (e.g., neighborhoods, road crossings, population centers, etc.), but these are unlikely to be the only habitable areas present within a species range. Another consideration is the accuracy of the reported WCT occurrences. Overall, 33 (70.2%) historic occurrence records originated over 50 years ago, when readily-available handheld electronic GPS units did not exist. Many of these coordinates were retroactively determined based on sometimes broad or generalized location descriptions. As a result, historic accounts could be inaccurate in the exact location WCT were observed, ultimately affecting MaxEnt habitat predictions. A third consideration is that, even if all coordinates were accurate, land cover data from 2001 (the oldest dataset available when models were performed) may not be truly representative of the habitat available in the early- to mid-20th century. Major changes in land cover prior to 2001, especially around urbanized city centers, may cause for inaccurate model outputs if those areas had not been previously urbanized at the time the observation was recorded. For example, if an occurrence reported in 1931 originated from a wetland habitat, urbanization over the course of 70 years could have spread into that area and significantly altered the land cover type documented in the 2001 NLCD. Though we cannot make direct inferences due to the lack of historic land cover data, these considerations align with previous reports suggesting that wetland loss from urbanization is likely the largest threat to WCT in Texas (Ryberg et al., 2016, 2017).

Unlike the historic SDM, the current SDM predicted high habitat suitability in areas not directly associated with city centers, going so far as to indicate that city centers and major highways were predicted to have the least suitable habitat for WCT. Conversely, the most suitable habitat occurred around urban fringes, with the majority of predicted habitat residing in the southeastern coastal plain and in low-lying areas of major river basins in central- and northeast Texas. Visual inspection further showed that more suitable habitat was often predicted in agricultural and wetland NLCD classes (e.g., Hay/Pasture, Woody Wetlands, Emergent Herbaceous Wetlands). Euclidean distance between Freshwater Emergent and Freshwater Pond wetlands was heavily relied on in the model prediction and wetlands clustered together were favored based on visual inspection, although we did not include other wetland classifications in the model tests. Freshwater emergent wetlands and ponds often form in low-lying areas near rivers or streams as streamflow fluctuates and, ultimately, floods surrounding areas. Additionally, jackknife results for AUC were variable with elevation causing the largest increase in model AUC when considered by itself, but distance between wetlands contained the most information not found in other co-variates. Visual inspection showed that higher suitability often occurred within the floodplains of major streams visible in the DEM. Areas with some of the lowest suitability occurred along the banks of larger streams where overall slope was greatest, despite this co-variate being one of the least important to the model. This suggests that large rivers and streams may serve as geographic barriers to WCT dispersal to nearby wetlands and may be an important consideration in future conservation efforts for the species.

In the detection SDM, which included only the 12 locations at which WCT were confirmed in the current study, slope, elevation, and soil class were identified as the most important co-variates, representing over 65% combined contribution to the model. Visual comparison of the detection and current SDMs show convergence in areas where more suitable habitat was predicted overall. However, there are fewer "hotspots" of habitat with high suitability (> 0.5) in the detection SDM. This is likely a product of the small sample size and lower WCT prevalence value (0.182) in comparison to the default (0.5) used in the other two models. Moderately suitable habitat was predicted along smaller rural roads in some areas. Although the sites sampled in this study were randomly generated based on proximity to preferred wetland types, reliance on historic occurrences may have still caused some bias toward roadways in the overall model. Visual inspection confirmed that the most suitable habitat areas in the detection SDM often matched HSG Group A. These soils typically have over 90% sand or gravel, less than 10% clay, sandy or gravelly textures, and low runoff potential when wet (USDA, 2007). While this indicates that WCT may be associated with sandy soils at the land-scape scale, small-scale habitat results support presence in areas where soils also contain clay components. Further assessment of the specific soil type(s) used by WCT is needed in order to definitively determine which type(s) are more or less important for the species, especially in relation to nesting, brumation, burrowing, etc. The detection SDM also follows a similar pattern to the current SDM in that some of the least suitable areas occurred where slope, the most important co-variate, was highest along streambanks.

Similar to the historic SDM, the current SDM may also contain intrinsic bias based on the locations of historic occurrences. For example, more suitable habitat was frequently predicted on roads (e.g., in easily accessible areas). Additionally, there may have been residual issues with accuracy from occurrences at the beginning of the current SDM timeframe (e.g., in early 2000) or accuracy issues from more recent occurrences that were obscured but not noted as obscured. As with the historic SDM, occurrences of temporal conflictions between the habitat type when

WCT were observed and the land cover class produced in the NLCD 2019 dataset may have also led to bias within the model. Though these biases are fairly unavoidable, we attempted to alleviate for them by including areas from the current study where WCT were potentially detected and confirmed. Potential detections also hold a level of bias in that, without a confirmed occurrence in an area, we cannot be completely sure that the location was inhabited by WCT at the time of sampling. Inclusion of these potential detection locations to the model allowed for increased spatial distribution of model input coordinates, ultimately aiding to increase the power of the model output. While we recognize that the output of the current SDM may include biases that cannot be alleviated, we believe that this model represents the most current prediction for WCT distribution, especially considering convergence of the model with the detection SDM.

When considering how relative habitat suitability (e.g., probability of presence) was affected by changes in environmental co-variates, there was a large amount of variability between the models. In general, the highest probabilities for each co-variate in the historic and current SDM were above 0.55, while those in the detection SDM were below 0.40, though this may be a byproduct of the smaller sample size in the detection SDM. For the current SDM, response curves showed overall negative relationships between continuous co-variates and habitat suitability, as indicated by the highest logistic probabilities being associated with smaller environmental values. For example, probability of detection dropped below the 0.3 threshold as elevation, topographic slope, and distance between wetlands increased. This trend also held true for the historic and detection SDM response curves, with the exception of road density for the historic model where suitability increased with increasing density. Though all three SDM resulted in a range of categorical co-variates showing best probability of detection, raw land cover class 21 (Developed, Open Space) and soil classes 1 and 7 (Group A and Group C/D, respectively) resulted in habitat probability of detection being above the 0.3 threshold across all models. Based on results of the SDM outputs from the current study, we recommend future assessments use the following environmental conditions as guidelines for determining potential survey areas: distance between Freshwater Emergent or Pond wetlands < 500 m, elevation between 5.0-125 m, raw land cover classes 11 (Open Water), 21-24 (Developed), 31 (Barren Land), 41-43 (Forest), 52 (Shrub/Scrub), 81 (Pasture/Hay), 82 (Cultivated Crops), 90 (Woody Wetlands), and 95 (Emergent Herbaceous Wetlands), road density < 8.0 km/km^2, and topographic slope < 2.75 degrees. While our models suggest that WCT presence may be predicted by all seven HSG soil classes, further evaluation of this particular variable should be considered, as previous studies have suggested that WCT exhibit an affinity for sandier substrates (Ryberg et al., 2017; Bowers et al., 2021). It should be noted that other environmental co-variates (e.g., weather and climate data) and additional watershed characteristics were not included in SDM for the current study. Ecological relationships between these other co-variates and WCT occurrences should be evaluated in future studies to determine other landscape conditions under which the species presence may be predicted.

Across all models, elevation consistently ranked among the top three co-variates based on importance and contribution, with the exception of permutation importance to the detection SDM where slope (which was calculated from elevation values in the DEM) ranked highest overall. Results for all three SDM iterations indicated the majority land cover class was not informative to the model in comparison to other environmental co-variates and each model performed slightly better when it was excluded during testing. We included this co-variate as an attempt to explain variation in habitat selection based on an anticipated increased home range for the species (Bowers et al., 2021). After evaluating model performance with this calculated variable, we recommend that use of the raw NLCD cover type is the best predictor of WCT occurrence when this type of data is considered, unless most of the occurrences used are located directly on roads and urban land classes, which may ultimately cause bias in the model outputs. Given that raw land cover comprised nearly half the total percent contribution and permutation importance in the historic SDM (47% and 45.6%, respectfully), the model's heavy reliance on this co-variate could have obscured relationships between WCT occurrences and other co-variates. See Appendix H as an example of a "quick fix" of attempting to remove urban sampling bias for this SDM. Finally, the distribution map for the current SDM suggests that WCT are more likely to reside in low-lying areas affiliated with the coastal plain of southeast Texas and wetlands in associated low-lying areas of major river basins in central-east and northeast Texas. Based on our results for small- and landscape scale habitat associations, WCT prefer small, ephemeral wetlands associated with Freshwater Emergent and Pond NWI classifications. Though wetted survey areas for Freshwater Emergent and Pond wetlands averaged 3.79 and 8.13 ha (37,900 and 81,300 m^2), respectively, our small-scale habitat data were collected within at 10 m x 10 m (100 m^2) assessment area at every site. Conversely, spatial resolution for all landscape scale co-variates was 30 m x 30 m (1 arc-second length; 900 m^2), which represents the finest resolution available from the raw raster datasets. Due to the variation in resolution between the two habitat analyses, location data used in the SDM may occur in cells with data classified differently than the smaller wetland habitat type within the same cell. This emphasizes the importance of analyzing habitat data at both levels (small- and landscape scale) in order to make best recommendations for future conservation efforts.

Efficacy and Efficiency of Survey Protocols

Though we were successful in detecting WCT using 13 of the 14 (92.9%) protocols applied in the current study, some protocols were more efficient and effective than others. Across all protocols, WS were the only method that did not yield detection(s) of WCT. While WS can be physically strenuous on the individual searcher, this survey protocol can be useful in detection of target species, especially within areas already know to be occupied by the target species (Gordon, *unpublished data*). In general, WS are less destructive to habitat than other protocols (e.g., trapping surveys), can ultimately result in capture or collection of the target species, and do not require as many personnel as other detection protocols (e.g., drone surveys or trapping surveys). Additionally, WS can be conducted in multiple targeted habitats, allowing the searcher(s) to methodically search a wide range of microhabitats within a given area, as opposed to other protocols which may be more restricted in the type of habitat(s) to which they can be applied. For example, geographic coverage for eDNA, BAVS, hoop trap, and camera trap protocols ranged from < 0.01% to 1.93% of the available habitat while geographic coverage for WS was 5.24%. In the current study, WS may not have been effective in detecting WCT because, while this is primarily a terrestrial survey method, it was primarily applied during the in-season (e.g., aquatic activity season) period, when WCT are more likely to reside in aquatic habitats. Efficiency of WS can be decreased in shallow aquatic habitats, like ephemeral wetlands, due to multiple factors. Primarily, creation of a sediment plume around the searcher reduces overall visibility in the water and allows the observed individual an opportunity to escape or hide within the plume. Secondly, glare on the water's surface occurring during surveys conducted later in the day can reduce overall visibility in the area around a searcher. Though this can be mitigated for by using polarized sunglasses and a hat, distortion of individuals from increased glare can make identification difficult. Additionally, restricted movement speeds of the individual searcher due to difficulties traversing aquatic or wetted habitats may ultimately lead to the searcher being unable to reach an observed individual prior to a confident species identification. A confounding factor to this restricted movement speed for the searcher is that, for most aquatic species, the target species ability to navigate aquatic environments is much improved over their ability to navigate terrestrial habitats. Though we do not recommend using WS as a protocol for initial detection of a target species in a new area or area without confirmed occupancy, we would still recommend its application in studies targeted at capturing individuals (e.g., population assessments), especially in areas where the target species is already known to occur. Furthermore, addition of detector dogs to traditional WS, as with our CSS surveys, improves the ability for detection of cryptic species and overall geographic area that can be covered. Addition of detector dog(s) increased detection probability from 0.000 to 0.007 and geographic coverage from 5.24% to over 92% of the given survey area. Detector dogs are able to search within multiple habitat types, many times accessing locations otherwise inaccessible to humans (e.g., thorny underbrush, areas comprised of softer substrate that a human may readily sink in to, etc.) (Cabalk and Heaton, 2006; Hoffman, 2014; Jean-Marie et al., 2019). While we were only able to apply CSS to a small handful of locations as part of a pilot study in this assessment, we recommend further evaluation of CSS as a viable method for detecting cryptic and hard to find species, like the WCT.

Of the protocols with multiple application types (eDNA and drone), some applications were more efficient and effective than others. For eDNA, A-3.0 and R-3.0 resulted in higher detection calculations (detection probability, "catch" per unit effort, and detection proportion) than the other three eDNA protocols. While no significant differences were detected in overall sub-category ranks for all eDNA protocols, A-3.0 and R-3.0 showed the greatest positive deviation from the mean, suggesting use as a best-recommended application in future surveys for this detection protocol. The M2 drone platform resulted in higher detection calculations and was ranked significantly better across all comparison rubric sub-categories than the P4 platform. Additionally, the M2 drone platform showed the greatest positive deviation from the mean, suggesting use as a best-recommended application in future surveys for this detection protocol. It should be noted that the cumulative and average rank for A-3.0 and R-3.0 eDNA protocols were significantly lower than both drone protocols, suggesting that eDNA protocols are better suited for detection than drone protocols. Additionally, for all sites with positive eDNA detections, WCT presence was confirmed using another protocol. Ultimately, application of a given protocol will depend on the over-arching goal or question of future assessments. For example, if the goal of a study is to detect WCT without visual confirmation, then A-3.0 and R-3.0 eDNA protocols would be recommended over drone protocols. Conversely, if the goal of a study is to detect WCT with visual confirmation, then the M2 protocol is recommended above the P4 and all eDNA protocols.

While our efforts resulted in multiple detections of WCT, total number of detections for each protocol were low. Total number of detections varied between protocols (range: 1-28) and the proportion of detections (number of events with confirmed WCT detections divided by total number of events for protocol) did not exceed 25% for any given protocol. Protocols with the highest detection proportion (R-3.0: 24.56%; A-3.0: 24.35%; ORT: 20.41%; Hoop trap: 18.18%) varied in calculated "catch" per unit effort (CPUE) (0.9825, 0.9739, 0.0204, and 0.0018, respectively). While some of the variation between these CPUE values may be explained by the way in which they were calculated, as standardization of catch rates for protocols not resulting in physical capture (e.g., eDNA) to protocols resulting in physical capture (e.g., trapping) is difficult, we believe the relative comparability of these CPUE calculations is accurate. For example, eDNA sample collection does not require much time or equipment in the field. While overall sample processing and analyses in the lab may be conducted over a cumulative total of a day or more, this additional "effort" is still less than the 24-hour, multi-day monitoring that is required for hoop trap surveys. With a cryptic and classically difficult to capture species, hoop trap surveys also resulted in fewer overall number of WCT captures when compared to the total number of eDNA samples that were collected and analyzed in less time. Should this have been a study focused on detection of the Red-eared Slider (*Trachemys scripta elegans*), a species that occurs in much higher concentrations across a wider variety of habitats, we would expect CPUE for trapping surveys to match, or even exceed, the calculated CPUE for eDNA protocols. Additionally, while the ORT requires an increased amount of time (e.g., effort) on the front end, once launched and advertised, it essentially serves as a passive data collection method which require minimal time during deployment to send out reminders or additional advertisements.

During year 1 of the study, much of the survey area experienced a prolonged drought, with one location in particular being completely dry during all sampling events. Additionally, in year 2, most of the survey area experienced above normal rainfall when compared to the 30-year normal (Gordon et al., 2021b). Increase in precipitation may have caused deviations from "normal" water quality levels and ultimately may have affected detection rates for all survey methods. For example, these climatic events may have impacted the persistence of genetic material due to increased exposure of soils to ultraviolet (UV) radiation during drought conditions or dilution, increased levels of inhibiting compounds, or alterations to water quality which may affect eDNA residency rates during flood conditions (Barnes and Turner, 2016; Seymour et al., 2018; Stewart, 2019). Additionally, areas inhabited by WCT may have been difficult to access during flood conditions, ultimately leading to non-detections because of inaccessibility to more suitable habitat. Another consideration is that, during extreme climatic events, individual WCT may have restricted activities or movements by entering a prolonged aestivation by burrowing in upland habitats or prolonged residency in a given waterbody with limited movement between habitats. Conversely, increased interconnectivity of wetland habitats may have cause individuals to increase movements between wetlands, ultimately reducing the overall concentration of eDNA present in a given wetland which may have been further compounded by a dilution effect from increased precipitation. This variation in overall environmental conditions between all three years of the survey may have ultimately impacted overall detection rates for all protocols applied. Further evaluation of protocols is needed across a more standardized window of environmental conditions in order to ensure that results from this study were not affected by variation in environmental conditions.

When protocols were compared through a series of hypothetical scenarios, different scenarios resulted in different best-recommendations for protocol application. For example, when the hypothetical goal was capture of individuals (e.g., for a population assessment), only two protocols could be compared – CSS and hoop trap surveys – as they were they only protocols to result in physical capture of individuals. Of these two, hoop trap surveys are the best-recommended protocol. Conversely, when the hypothetical goal of a future assessment was to detect individuals without concern for costs, R-3.0 and A-3.0 protocols were recommended over all others. Finally, when the hypothetical goal of a future assessment was to detect individuals using protocols with the lowest costs, a combination of RS, BAVS, A-3.0, and R-3.0 protocols was recommended. While we can make best recommendations for which protocols to apply in a given scenario, our primary recommendation is to apply a combination of sampling techniques, regardless of question or over-arching goal, in order to maximize efficiency and effectiveness for assessment of this cryptic and wide-spread species in Texas.

Recommendations and Future Research Needs

Further analysis of small-scale habitat preferences of WCT, their relation to macro-scale ecological factors, and how anthropogenic factors may threaten the availability of each are needed in areas not covered by the current study. Determination of habitat heterogeneity found within the typical distance WCT travel in a year could provide insight into annual ecological factors WCT require for life history functions beyond the microhabitat scale sampled in this study. Existing habitat fragmentation analyses (such as those used in Ryberg et al. 2016) could be expanded upon to assess the connectivity of the suitable WCT habitat using updated distribution models from this study.

In addition to the environmental co-variates we assessed in SDM, future SDM for the WCT should focus on inclusion of other co-variates to the model(s). Inclusion of past and current weather data grids could be used to indicate the temperature and precipitation preferences of or limitations to WCT as activity and wetland availability are dependent on these factors. Future projections of WCT distribution in Texas based on a changing environment (eco-forecasting) could be made using climate change projections from the Intergovernmental Panel on Climate Change or other sources.

While we were able to compare protocols applied in the current study using a standardized comparison rubric, additional conservation considerations should be examined for refinement of the rubric. Level of disturbance or destruction to the habitat using a given protocol, inevitable stress or risk of injury to the target species or by-catch, and potential for introduction of invasive species, zoonotic diseases, etc. should all be considered when deciding to apply a protocol, especially in a large survey area, such as east Texas.

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Appendix A – Final Report for Supplemental (SRA) Sites

ELECTRONIC SUPPLEMENT

File Name: 20-6997BG_Western Chicken Turtle_Final Report_Appendix A

Description: Full copy of final report submitted to the Sabine River Authority of Texas per requirements of supplemental contract 2021-001. Includes preliminary analyses for four supplemental sites sampled using environmental DNA (eDNA), the Mavic 2 Enterprise Dual drone ($Drone_{M2}$), and binocular assisted visual survey (BAVS) methods. Data from these four sites will be incorporated into the final project in Year 3.

Appendix B – Historic Occurrences Used in Randomized Site Generation and SDM

This appendix includes a full list of the 103 publicly available spatial occurrence data for Western Chicken Turtles (*Deirochelys reticularia miaria*) used in site selection (including randomized site generation) and species distribution models (SDM).

Appendix Table B.1 List of the historic occurrence coordinates (*n* = 103) used in randomized site generation and species distribution models (SDM). Data were extracted from VertNet (accessed 06 January 2020), iNaturalist (accessed 26 January 2020), the Global Biodiversity Information Facility (GBIF; accessed 25 October 2022), literature (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019), and communications with experts in the field. Includes data source, latitude and longitude, species reported, specimen type, observation date, county, notes about coordinate obscuring, and in which application the coordinates were used. Occurrences from 1922-2000 were used in "historic" SDM and those from 2001-2022 were used in "current" SDM.

Source	Latitude	Longitude	Species reported	Specimen type	Date	County	Obscured	Application
VertNet, 2020	29.53098	-96.57396	*Deirochelys reticularia*	Preserved specimen	08/01/1922	Colorado	Unknown	Site selection, SDM
VertNet, 2020	33.17890	-95.36040	*Deirochelys reticularia*	Preserved specimen	05/02/1931	Hopkins	Unknown	Site selection, SDM
VertNet, 2020	32.81381	-96.72680	*Deirochelys reticularia miaria*	Preserved specimen	04/16/1932	Dallas	Unknown	SDM
VertNet, 2020	32.81370	-96.72670	*Deirochelys reticularia miaria*	Preserved specimen	04/16/1932	Dallas	Unknown	Site selection
VertNet, 2020	29.53235	-96.44358	*Deirochelys reticularia*	Preserved specimen	05/19/1938	Colorado	Unknown	Site selection, SDM
VertNet, 2020	30.86224	-96.94328	*Deirochelys reticularia*	Preserved specimen	07/11/1940	Milam	Unknown	Site selection, SDM
VertNet, 2020	30.62778	-96.33484	*Deirochelys reticularia*	Preserved specimen	05/01/1941	Brazos	Unknown	Site selection, SDM
VertNet, 2020	29.45410	-95.91829	*Deirochelys reticularia*	Preserved specimen	07/15/1946	Fort Bend	Unknown	Site selection, SDM
VertNet, 2020	29.45338	-95.92167	*Deirochelys reticularia*	Preserved specimen	07/15/1946	Fort Bend	Unknown	Site selection
VertNet, 2020	30.19272	-94.62588	*Deirochelys reticularia*	Preserved specimen	04/10/1948	Liberty	Unknown	Site selection, SDM
VertNet, 2020	32.70728	-96.73618	*Deirochelys reticularia*	Preserved specimen	05/16/1948	Dallas	Unknown	Site selection, SDM
VertNet, 2020	30.01450	-95.48096	*Deirochelys reticularia*	Preserved specimen	03/19/1949	Harris	Unknown	Site selection, SDM
VertNet, 2020	29.11828	-96.83920	*Deirochelys reticularia*	Preserved specimen	07/17/1949	Lavaca	Unknown	Site selection, SDM
VertNet, 2020	30.61333	-96.33444	*Deirochelys reticularia*	Preserved specimen	07/31/1949	Brazos	Unknown	Site selection, SDM
VertNet, 2020	30.39054	-95.98283	*Deirochelys reticularia*	Preserved specimen	03/18/1950	Grimes	Unknown	Site selection, SDM
VertNet, 2020	32.71212	-94.12128	*Deirochelys reticularia*	Preserved specimen	08/01/1950	Harrison	Unknown	Site selection, SDM
VertNet, 2020	29.96328	-94.34429	*Deirochelys reticularia*	Preserved specimen	05/10/1952	Jefferson	Unknown	Site selection, SDM
VertNet, 2020	30.46697	-96.61079	*Deirochelys reticularia*	Preserved specimen	03/03/1956	Burleson	Unknown	Site selection, SDM
VertNet, 2020	29.56383	-95.02583	*Deirochelys reticularia*	Preserved specimen	03/22/1957	Harris	Unknown	Site selection, SDM
VertNet, 2020	29.27039	-95.32517	*Deirochelys reticularia*	Preserved specimen	04/01/1957	Brazoria	Unknown	Site selection, SDM
VertNet, 2020	29.87367	-94.19400	*Deirochelys reticularia*	Preserved specimen	04/22/1957	Jefferson	Unknown	Site selection, SDM
VertNet, 2020	29.49750	-95.95139	*Deirochelys reticularia*	Preserved specimen	04/15/1958	Fort Bend	Unknown	Site selection, SDM
VertNet, 2020	29.69250	-94.62889	*Deirochelys reticularia*	Preserved specimen	04/05/1959	Chambers	Unknown	Site selection, SDM
VertNet, 2020	30.73250	-96.37000	*Deirochelys reticularia*	Preserved specimen	06/22/1961	Brazos	Unknown	Site selection, SDM
VertNet, 2020	30.08702	-95.76324	*Deirochelys reticularia*	Preserved specimen	08/13/1961	Harris	Unknown	Site selection, SDM
VertNet, 2020	30.06617	-95.45513	*Deirochelys reticularia*	Preserved specimen	09/13/1961	Harris	Unknown	Site selection, SDM
VertNet, 2020	29.60194	-94.67528	*Deirochelys reticularia*	Preserved specimen	02/23/1962	Chambers	Unknown	Site selection, SDM
VertNet, 2020	29.47297	-96.07144	*Deirochelys reticularia*	Preserved specimen	05/07/1964	Wharton	Unknown	Site selection, SDM
VertNet, 2020	30.61778	-96.33722	*Deirochelys reticularia*	Preserved specimen	05/10/1964	Brazos	Unknown	Site selection, SDM
VertNet, 2020	31.96429	-96.62474	*Deirochelys reticularia*	Preserved specimen	04/14/1965	Navarro	Unknown	Site selection, SDM
VertNet, 2020	30.00463	-95.31183	*Deirochelys reticularia*	Preserved specimen	04/06/1966	Harris	Unknown	Site selection, SDM
VertNet, 2020	29.90857	-95.95786	*Deirochelys reticularia*	Preserved specimen	04/15/1967	Waller	Unknown	Site selection, SDM

Appendix Table B.1 List of the historic occurrence coordinates (*n* = 103) used in randomized site generation and species distribution models (SDM). Data were extracted from VertNet (accessed 06 January 2020), iNaturalist (accessed 26 January 2020), the Global Biodiversity Information Facility (GBIF; accessed 25 October 2022), literature (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019), and communications with experts in the field. Includes data source, latitude and longitude, species reported, specimen type, observation date, county, notes about coordinate obscuring, and in which application the coordinates were used. Occurrences from 1922-2000 were used in "historic" SDM and those from 2001-2022 were used in "current" SDM.

Source	Latitude	Longitude	Species reported	Specimen type	Date	County	Obscured	Application
VertNet, 2020	30.67618	-93.94486	*Deirochelys reticularia*	Preserved specimen	04/29/1967	Jasper	Unknown	Site selection, SDM
VertNet, 2020	29.40903	-95.04704	*Deirochelys reticularia*	Preserved specimen	04/11/1968	Galveston	Unknown	Site selection, SDM
VertNet, 2020	29.69664	-94.37432	*Deirochelys reticularia*	Preserved specimen	05/12/1968	Chambers	Unknown	Site selection, SDM
VertNet, 2020	30.08049	-96.39802	*Deirochelys reticularia*	Preserved specimen	03/25/1971	Austin	Unknown	Site selection, SDM
VertNet, 2020	32.41421	-96.13178	*Deirochelys reticularia*	Preserved specimen	04/15/1978	Kaufman	Unknown	Site selection, SDM
iNaturalist, 2020	32.58700	-96.30613	*Deirochelys reticularia miaria*	Online report	04/14/1982	Kaufman	No	Site selection, SDM
VertNet, 2020	32.06138	-95.55098	*Deirochelys reticularia miaria*	Preserved specimen	03/15/1983	Henderson	Unknown	Site selection, SDM
VertNet, 2020	32.91058	-96.27765	*Deirochelys reticularia miaria*	Preserved specimen	06/21/1984	Hunt	Unknown	Site selection, SDM
VertNet, 2020	32.89689	-95.90064	*Deirochelys reticularia miaria*	Preserved specimen	06/21/1984	Hunt	Unknown	Site selection, SDM
VertNet, 2020	33.32364	-95.79530	*Deirochelys reticularia*	Preserved specimen	06/22/1984	Delta	Unknown	Site selection, SDM
iNaturalist, 2020	32.87617	-96.26994	*Deirochelys reticularia*	Online report	05/11/1986	Hunt	No	Site selection, SDM
VertNet, 2020	31.84663	-96.44779	*Deirochelys reticularia*	Preserved specimen	03/15/1987	Navarro	Unknown	Site selection, SDM
VertNet, 2020	30.34250	-96.99167	*Deirochelys reticularia*	Preserved specimen	03/31/1989	Lee	Unknown	Site selection, SDM
VertNet, 2020	30.46190	-96.53257	*Deirochelys reticularia*	Preserved specimen	04/06/1989	Burleson	Unknown	Site selection, SDM
iNaturalist, 2020	29.55216	-94.38735	*Deirochelys reticularia*	Online report	04/06/1989	Chambers	No	Site selection, SDM
VertNet, 2020	30.21449	-95.76126	*Deirochelys reticularia miaria*	Preserved specimen	04/20/1991	Montgomery	Unknown	Site selection, SDM
iNaturalist, 2020	31.93605	-95.88767	*Deirochelys reticularia miaria*	Online report	03/26/2000	Anderson	No	Site selection, SDM
Adams and Saenz, 2011	31.49845	-94.77708	*Deirochelys reticularia miaria*	Live capture	05/01/2006	Nacogdoches	No	Site selection, SDM
iNaturalist, 2020	29.33894	-95.59779	*Deirochelys reticularia miaria*	Online report	03/27/2007	Fort Bend	No	Site selection, SDM
VertNet, 2020	29.95360	-95.89738	*Deirochelys reticularia*	Preserved specimen	04/05/2008	Waller	Unknown	Site selection, SDM
VertNet, 2020	30.46958	-96.21330	*Deirochelys reticularia*	Preserved specimen	08/07/2009	Brazos	Unknown	Site selection, SDM
iNaturalist, 2020	30.46922	-96.21151	*Deirochelys reticularia miaria*	Online report	08/09/2009	Brazos	No	Site selection
iNaturalist, 2020	30.46923	-96.21151	*Deirochelys reticularia miaria*	Online report	08/09/2009	Brazos	No	SDM
iNaturalist, 2020	30.75815	-96.73808	*Deirochelys reticularia miaria*	Online report	02/21/2010	Milam	Yes	Site selection
GBIF, 2022	30.02977	-95.29963	*Deirochelys reticularia miaria*	Online report	03/26/2011	Harris	No	SDM
Personal Communication	32.69241	-94.17962	*Deirochelys reticularia miaria*	Visual observation	05/01/2013	Harrison	No	SDM
VertNet, 2020	30.44683	-94.91416	*Deirochelys reticularia*	Preserved specimen	05/29/2013	Liberty	Unknown	Site selection, SDM
iNaturalist, 2020	30.44688	-94.91416	*Deirochelys reticularia*	Online report	05/29/2013	Liberty	Unknown	Site selection
Ryberg et al., 2017	31.12100	-96.92500	*Deirochelys reticularia miaria*	Visual observation	04/15/2015	Falls	Yes	Site selection
Ryberg et al., 2017	29.99500	-95.86100	*Deirochelys reticularia miaria*	Live capture	05/02/2015	Harris	Yes	Site selection
VertNet, 2020	29.87438	-95.86858	*Deirochelys reticularia*	Preserved specimen	05/03/2015	Waller	Unknown	Site selection
iNaturalist, 2020	29.88140	-95.98472	*Deirochelys reticularia*	Online report	05/03/2015	Waller	Unknown	Site selection
Ryberg et al., 2017	29.92300	-96.02900	*Deirochelys reticularia miaria*	Salvaged specimen	05/05/2015	Waller	Yes	Site selection
iNaturalist, 2020	29.81943	-95.97403	*Deirochelys reticularia miaria*	Online report	05/06/2015	Waller	Yes	Site selection
Personal Communication	32.69241	-94.17962	*Deirochelys reticularia miaria*	Visual observation	07/01/2015	Harrison	No	SDM
iNaturalist, 2020	29.86308	-95.88122	*Deirochelys reticularia*	Online report	04/03/2016	Waller	Unknown	Site selection
iNaturalist, 2020	29.94857	-95.85024	*Deirochelys reticularia miaria*	Online report	04/03/2016	Waller	Yes	Site selection
iNaturalist, 2020	31.27697	-96.43073	*Deirochelys reticularia*	Online report	04/27/2016	Robertson	No	Site selection, SDM

Appendix Table B.1 List of the historic occurrence coordinates (n = 103) used in randomized site generation and species distribution models (SDM). Data were extracted from VertNet (accessed 06 January 2020), iNaturalist (accessed 26 January 2020), the Global Biodiversity Information Facility (GBIF; accessed 25 October 2022), literature (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019), and communications with experts in the field. Includes data source, latitude and longitude, species reported, specimen type, observation date, county, notes about coordinate obscuring, and in which application the coordinates were used. Occurrences from 1922-2000 were used in "historic" SDM and those from 2001-2022 were used in "current" SDM.

Source	Latitude	Longitude	Species reported	Specimen type	Date	County	Obscured	Application
iNaturalist, 2020	30.47297	-96.22537	*Deirochelys reticularia*	Online report	06/28/2016	Brazos	No	Site selection, SDM
iNaturalist, 2020	30.47383	-94.22490	*Deirochelys reticularia*	Online report	05/14/2017	Hardin	No	Site selection, SDM
iNaturalist, 2020	31.48170	-94.76917	*Deirochelys reticularia*	Online report	03/01/2018	Nacogdoches	Yes	Site selection
iNaturalist, 2020	31.48941	-94.74770	*Deirochelys reticularia*	Online report	03/24/2018	Nacogdoches	No	Site selection, SDM
iNaturalist, 2020	29.70731	-95.90471	*Deirochelys reticularia miaria*	Online report	04/03/2018	Fort Bend	No	Site selection
Franklin et al., 2019	29.70723	-95.90473	*Deirochelys reticularia miaria*	Live capture	04/03/2018	Fort Bend	No	Site selection, SDM
iNaturalist, 2020	29.81729	-95.97282	*Deirochelys reticularia miaria*	Online report	04/07/2018	Waller	Unknown	Site selection
iNaturalist, 2020	29.88101	-95.92232	*Deirochelys reticularia miaria*	Online report	04/07/2018	Waller	No	Site selection, SDM
iNaturalist, 2020	29.93348	-95.92783	*Deirochelys reticularia*	Online report	04/09/2018	Waller	No	Site selection, SDM
iNaturalist, 2020	29.92697	-95.92406	*Deirochelys reticularia miaria*	Online report	05/29/2018	Waller	No	Site selection, SDM
iNaturalist, 2020	29.52534	-95.91138	*Deirochelys reticularia miaria*	Online report	03/11/2019	Fort Bend	No	Site selection, SDM
iNaturalist, 2020	31.49114	-94.74947	*Deirochelys reticularia miaria*	Online report	03/11/2019	Nacogdoches	No	Site selection, SDM
GBIF, 2022	29.40755	-94.63119	*Deirochelys reticularia miaria*	Online report	04/05/2019	Chambers	Yes	None; obscured
iNaturalist, 2020	29.96511	-95.89082	*Deirochelys reticularia miaria*	Online report	04/06/2019	Waller	Yes	Site selection
iNaturalist, 2020	29.93050	-95.99999	*Deirochelys reticularia*	Online report	04/06/2019	Waller	Unknown	Site selection
iNaturalist, 2020	29.97275	-95.97928	*Deirochelys reticularia*	Online report	04/06/2019	Waller	Unknown	Site selection
iNaturalist, 2020	31.58698	-94.61345	*Deirochelys reticularia*	Online report	04/10/2019	Nacogdoches	Yes	Site selection
iNaturalist, 2020	29.69916	-93.94895	*Deirochelys reticularia miaria*	Online report	04/16/2019	Jefferson	No	Site selection, SDM
iNaturalist, 2020	31.50711	-94.78701	*Deirochelys reticularia miaria*	Online report	04/27/2019	Nacogdoches	Yes	Site selection
iNaturalist, 2020	31.49149	-94.74774	*Deirochelys reticularia*	Online report	04/28/2019	Nacogdoches	No	Site selection, SDM
iNaturalist, 2020	31.41164	-94.79552	*Deirochelys reticularia*	Online report	04/28/2019	Nacogdoches	Unknown	Site selection
iNaturalist, 2020	29.66154	-94.62559	*Deirochelys reticularia miaria*	Online report	04/29/2019	Chambers	No	Site selection, SDM
GBIF, 2022	29.27979	-95.62296	*Deirochelys reticularia miaria*	Online report	07/03/2019	Brazoria	No	SDM
iNaturalist, 2020	32.67150	-94.10052	*Deirochelys reticularia*	Online report	07/10/2019	Harrison	No	Site selection, SDM
GBIF, 2022	29.90068	-94.05265	*Deirochelys reticularia miaria*	Online report	05/04/2020	Jefferson	No	SDM
GBIF, 2022	29.90748	-94.17547	*Deirochelys reticularia miaria*	Online report	05/16/2020	Jefferson	Yes	None; obscured
Personal Communication	32.99897	-94.43231	*Deirochelys reticularia miaria*	Visual observation	06/01/2020	Cass	No	SDM
GBIF, 2022	29.24080	-96.27339	*Deirochelys reticularia miaria*	Online report	07/03/2020	Wharton	No	SDM
GBIF, 2022	29.39016	-96.22134	*Deirochelys reticularia miaria*	Online report	04/08/2021	Wharton	Yes	None; obscured
GBIF, 2022	28.92843	-95.44156	*Deirochelys reticularia miaria*	Online report	05/24/2021	Brazoria	Yes	None; obscured
GBIF, 2022	29.74323	-93.94526	*Deirochelys reticularia miaria*	Online report	04/20/2022	Jefferson	Yes	None; obscured
GBIF, 2022	29.93352	-95.97497	*Deirochelys reticularia miaria*	Online report	04/20/2022	Waller	Yes	None; obscured
GBIF, 2022	29.91432	-95.84405	*Deirochelys reticularia miaria*	Online report	09/24/2022	Harris	Yes	None; obscured
VertNet, 2020	30.67437	-96.36989	*Deirochelys reticularia*	Preserved specimen	Unknown	Brazos	Unknown	Site selection
VertNet, 2020	30.65119	-94.10469	*Deirochelys reticularia*	Preserved specimen	Unknown	Tyler	Unknown	Site selection

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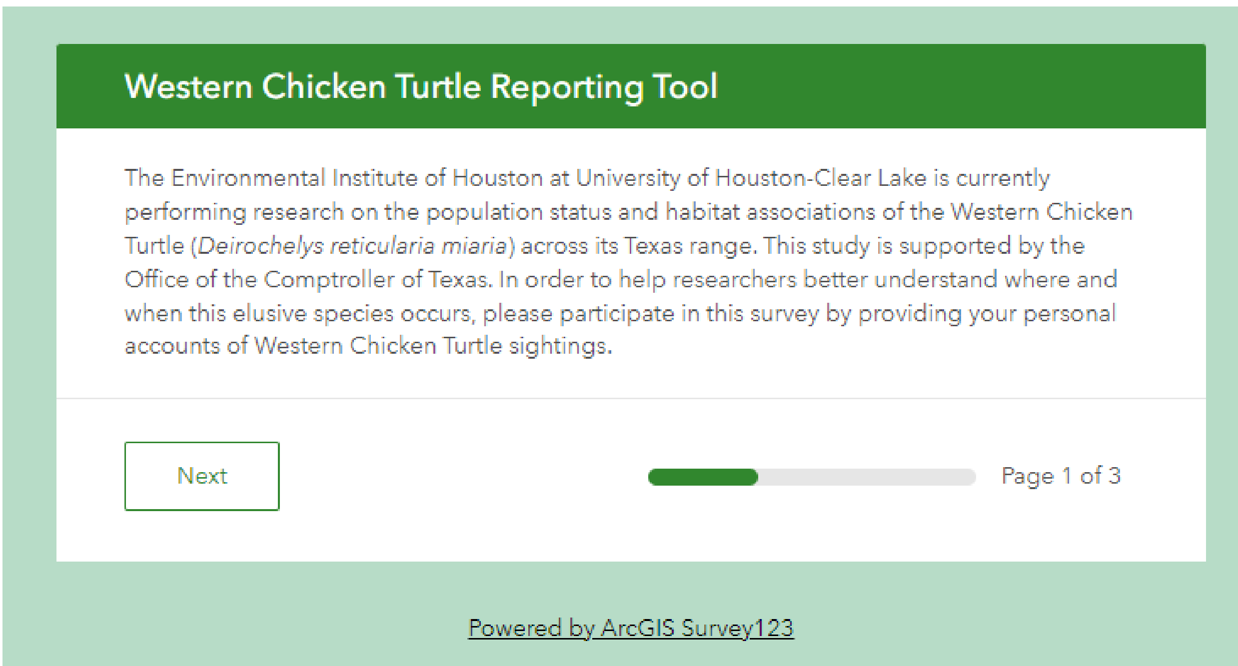
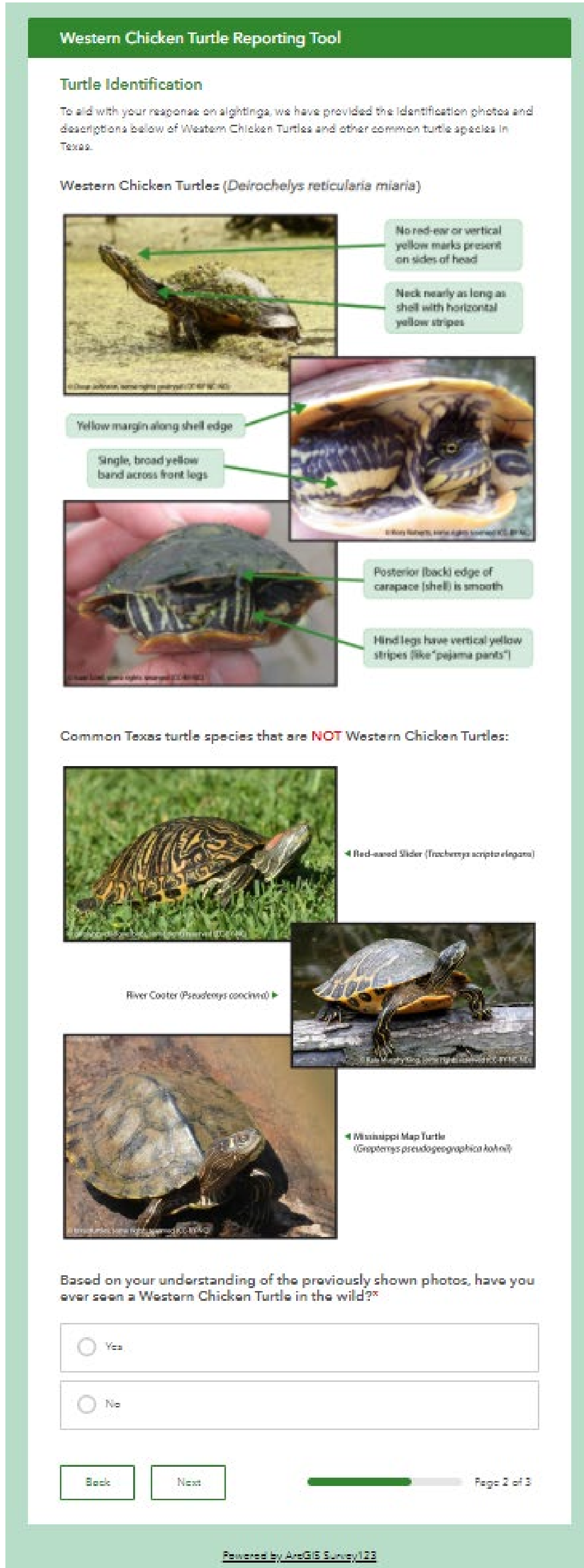
Appendix C– Online Reporting Tool Layout

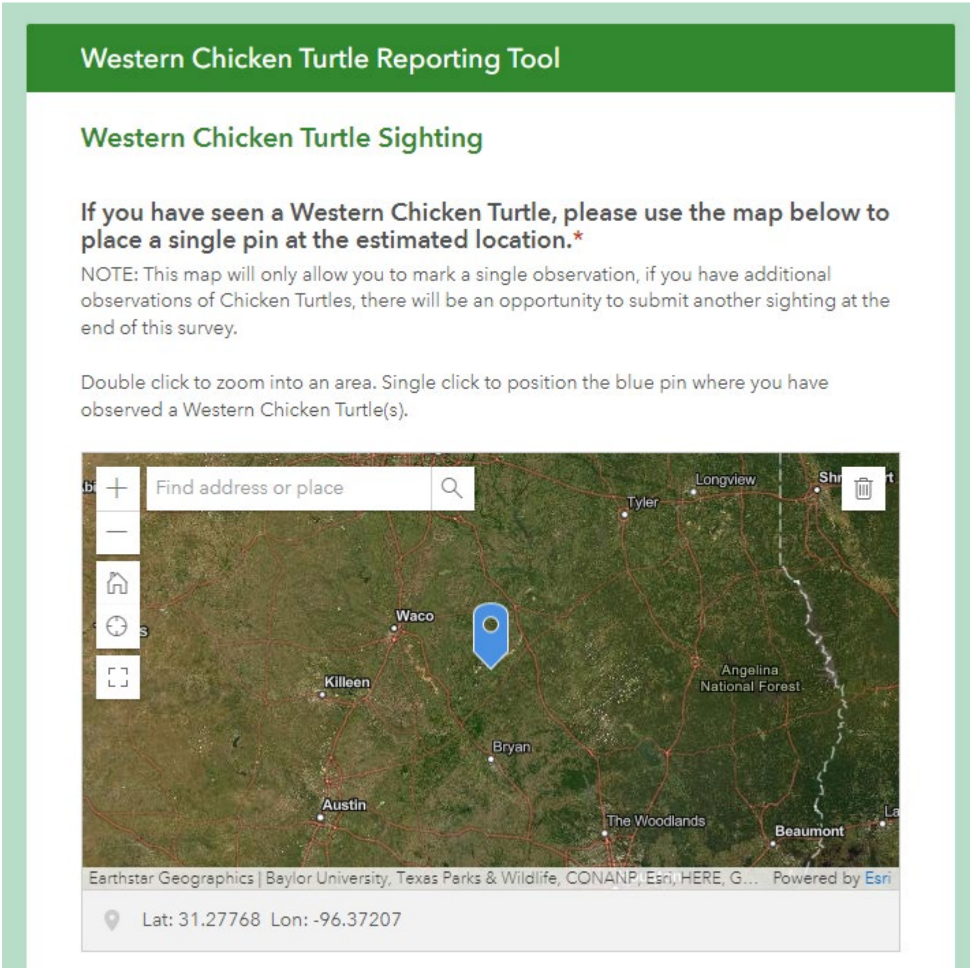
Below is a series of screenshots taken from the citizen-science based online reporting tool (ORT) used in Year 2 and Year 3 of the current study. Respondents were required to fill in most sections of the ORT and could provide contact information upon completion of the report but if a respondent opted to remain anonymous, they could. In each section of Page 3, field options would change based on the respondent's responses (see imagery below) and respondents were able to make multiple reports (if needed). Certain fields were required and the respondent could not complete the reporting tool unless these fields were completed. A progress scale was provided so the respondent could track the remaining duration of the reporting tool.



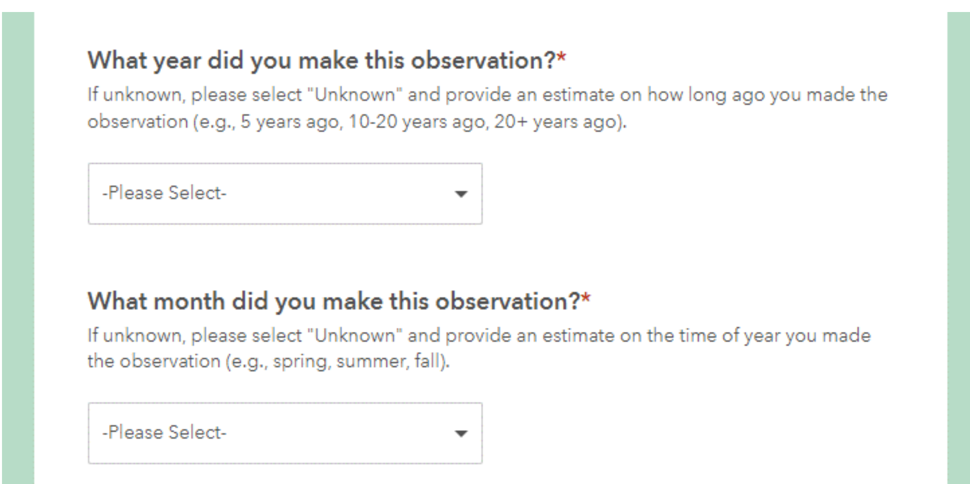
Appendix Figure C.1 Page 1 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This page provided background information for why the Reporting Tool was being established and informed the respondent of whom was conducting the data collection.



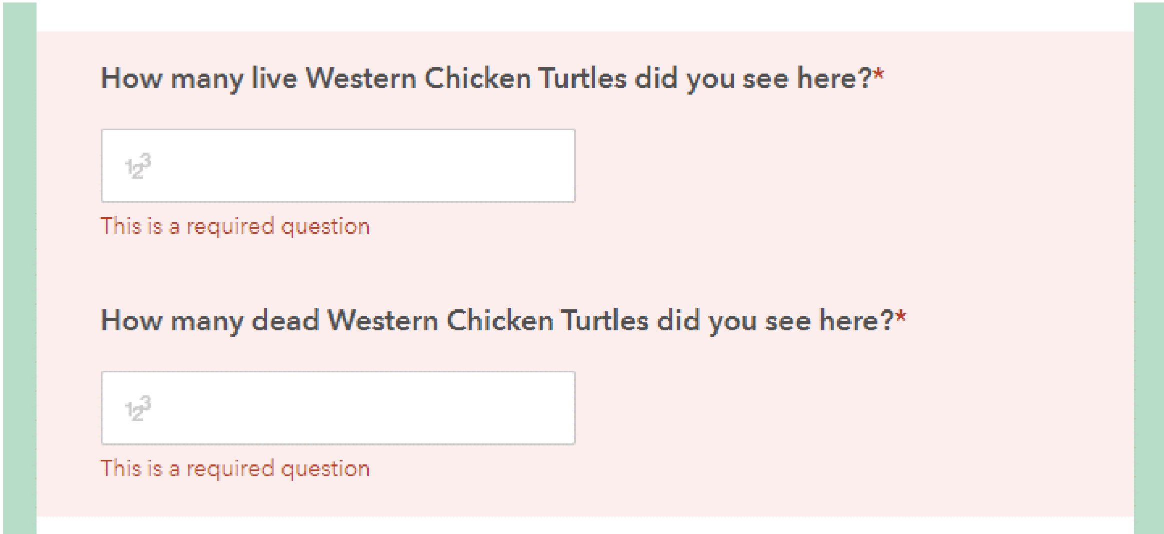
Appendix Figure C.2 Page 2 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This page required respondents to confirm their report was of a WCT. If the respondent selected "No", the survey ended.



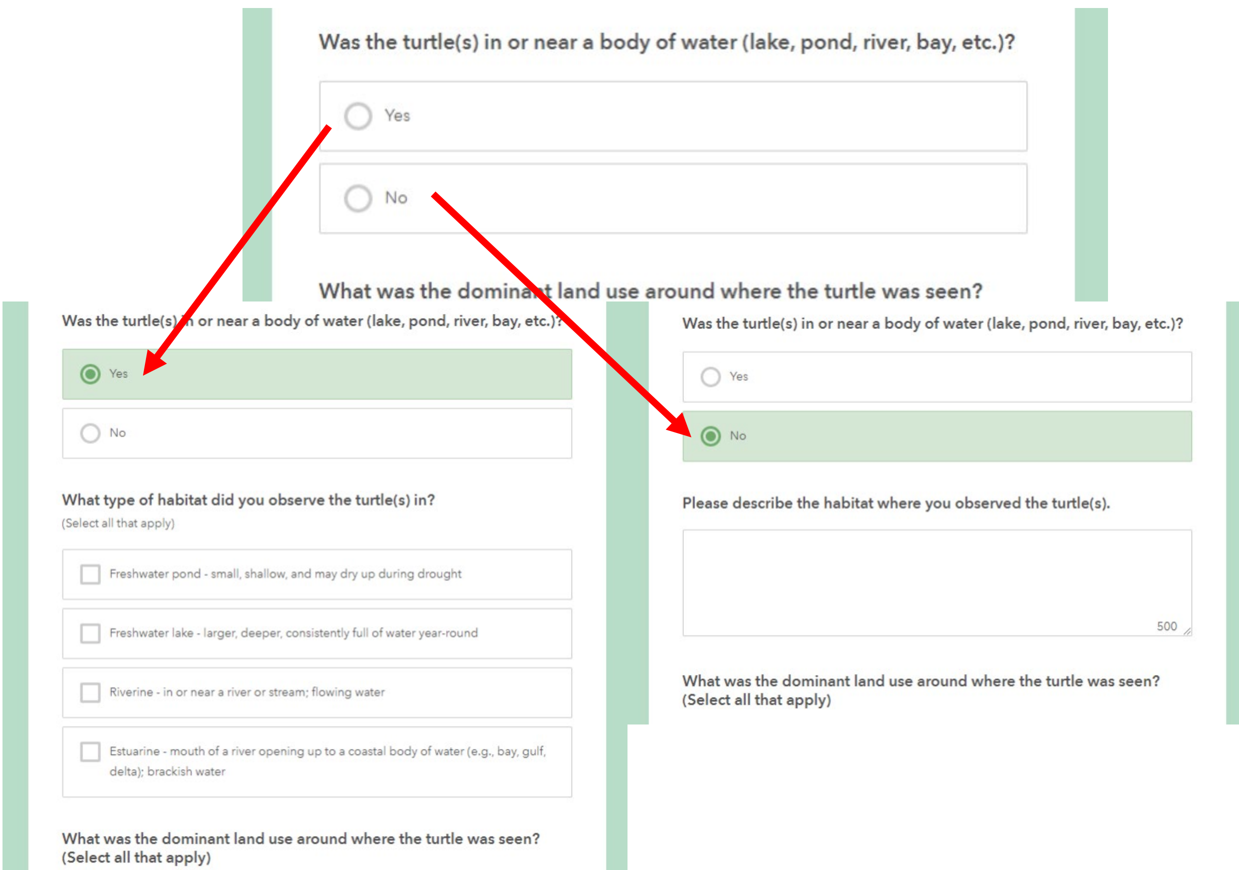
Appendix Figure C.3 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section of the page allowed the respondent to find the observation location by clicking, zooming, or panning on a map. The respondent could also enter a specific address or use a GPS coordinate to find and mark the observation location.



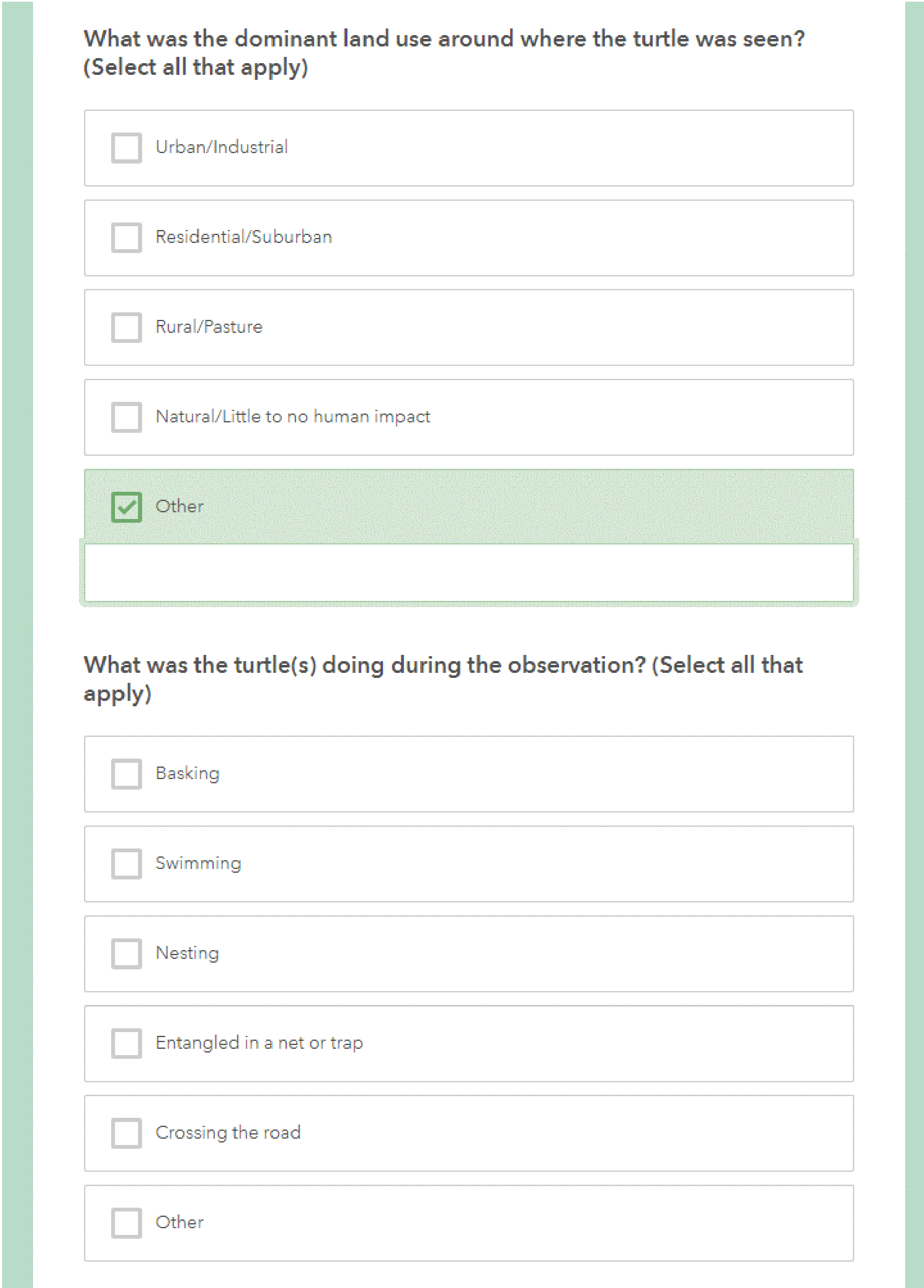
Appendix Figure C.4 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section allowed the respondent the opportunity to provide temporal information related to the observation.



Appendix Figure C.5 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section allowed the respondent the opportunity to provide counts of living or dead WCT observed. As with previous and future sections, this was required information and if the respondent failed to enter information in this section, the field was flagged, and the respondent could not move on to the next page without completing the section.



Appendix Figure C.6 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section allowed the respondent the opportunity to provide microhabitat information. As with future sections, follow up questions would change based on the response. Red arrows provide examples of how the subsequent questions would change based on the respondent's answer.



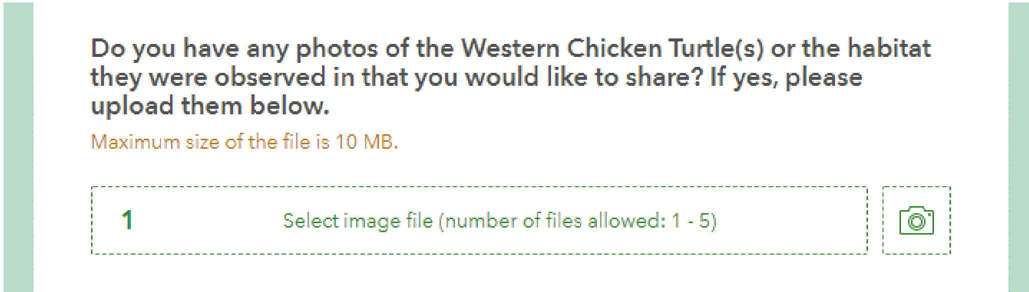
What was the dominant land use around where the turtle was seen? (Select all that apply)

- [ ] Urban/Industrial
- [ ] Residential/Suburban
- [ ] Rural/Pasture
- [ ] Natural/Little to no human impact
- [x] Other

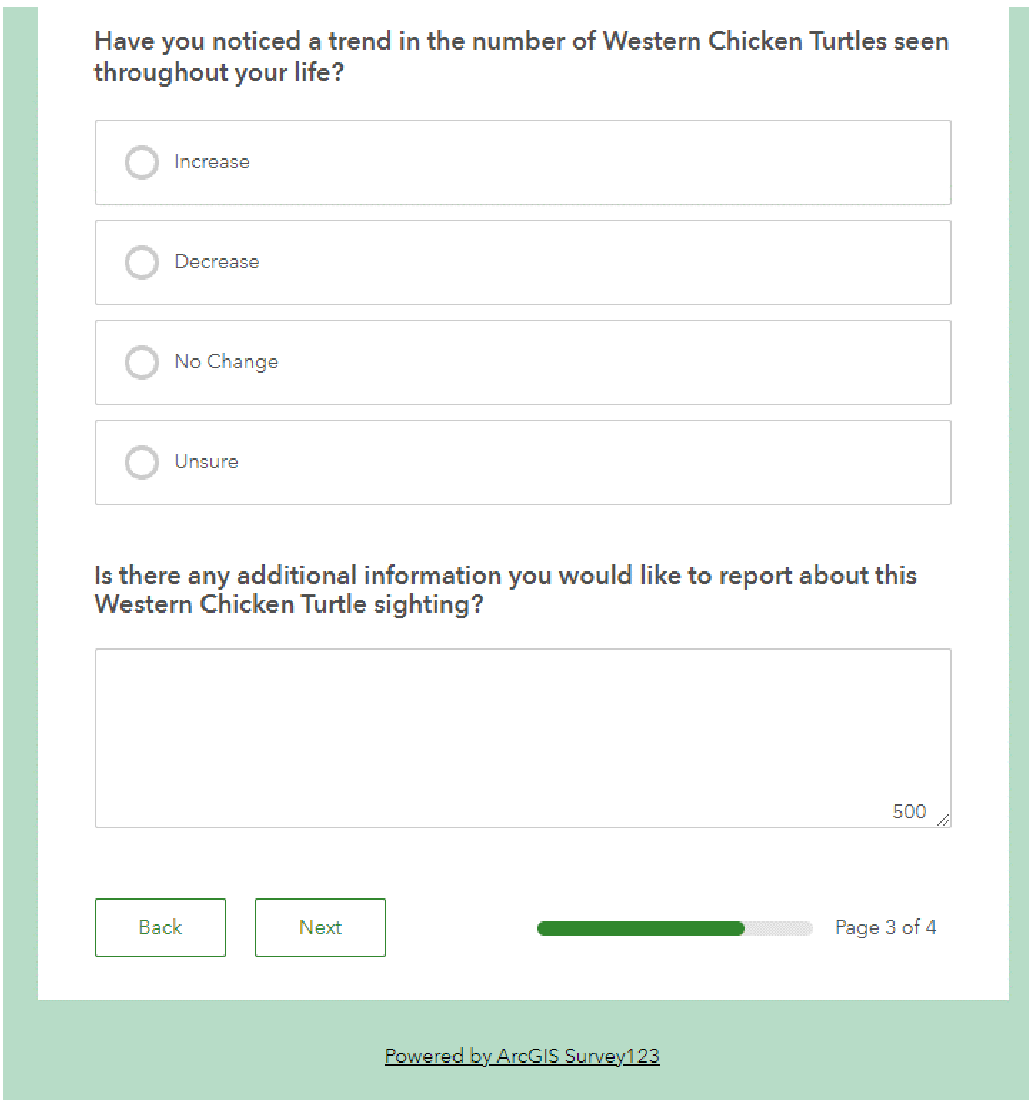
What was the turtle(s) doing during the observation? (Select all that apply)

- [ ] Basking
- [ ] Swimming
- [ ] Nesting
- [ ] Entangled in a net or trap
- [ ] Crossing the road
- [ ] Other

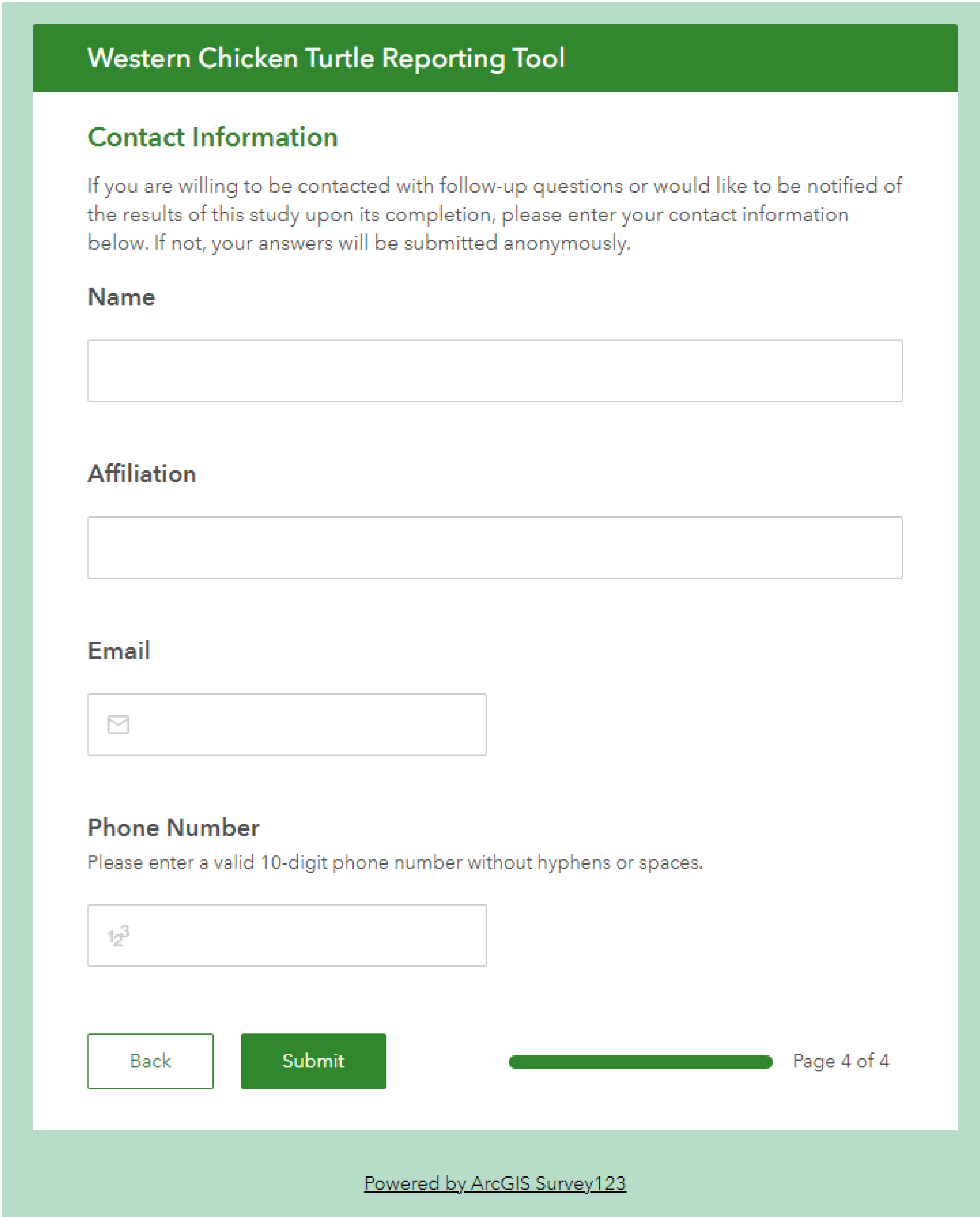
Appendix Figure C.7 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section allowed respondents the opportunity to provide macrohabitat information and behavior. As with previous and subsequent sections, if the respondent selected "Other", a character-limited text box would appear so the respondent could provide a brief description.



Appendix Figure C.8 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This section allowed the respondent the opportunity to provide photographs or other files related to the WCT observation. Respondents could provide up to 5 files (including map screenshots, PDF documents, photos of the individual, etc.) and maximum file size was limited to 10 MB.



Appendix Figure C.9 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This final section allowed the respondent the opportunity to provide perceived observations on general trends in number of WCT observed during their lifetime. It also allowed respondents to provide any other anecdotal information related to the observation. Anecdotal information had a 500-character limit.



Western Chicken Turtle Reporting Tool

Contact Information

If you are willing to be contacted with follow-up questions or would like to be notified of the results of this study upon its completion, please enter your contact information below. If not, your answers will be submitted anonymously.

Name

Affiliation

Email

Phone Number

Please enter a valid 10-digit phone number without hyphens or spaces.

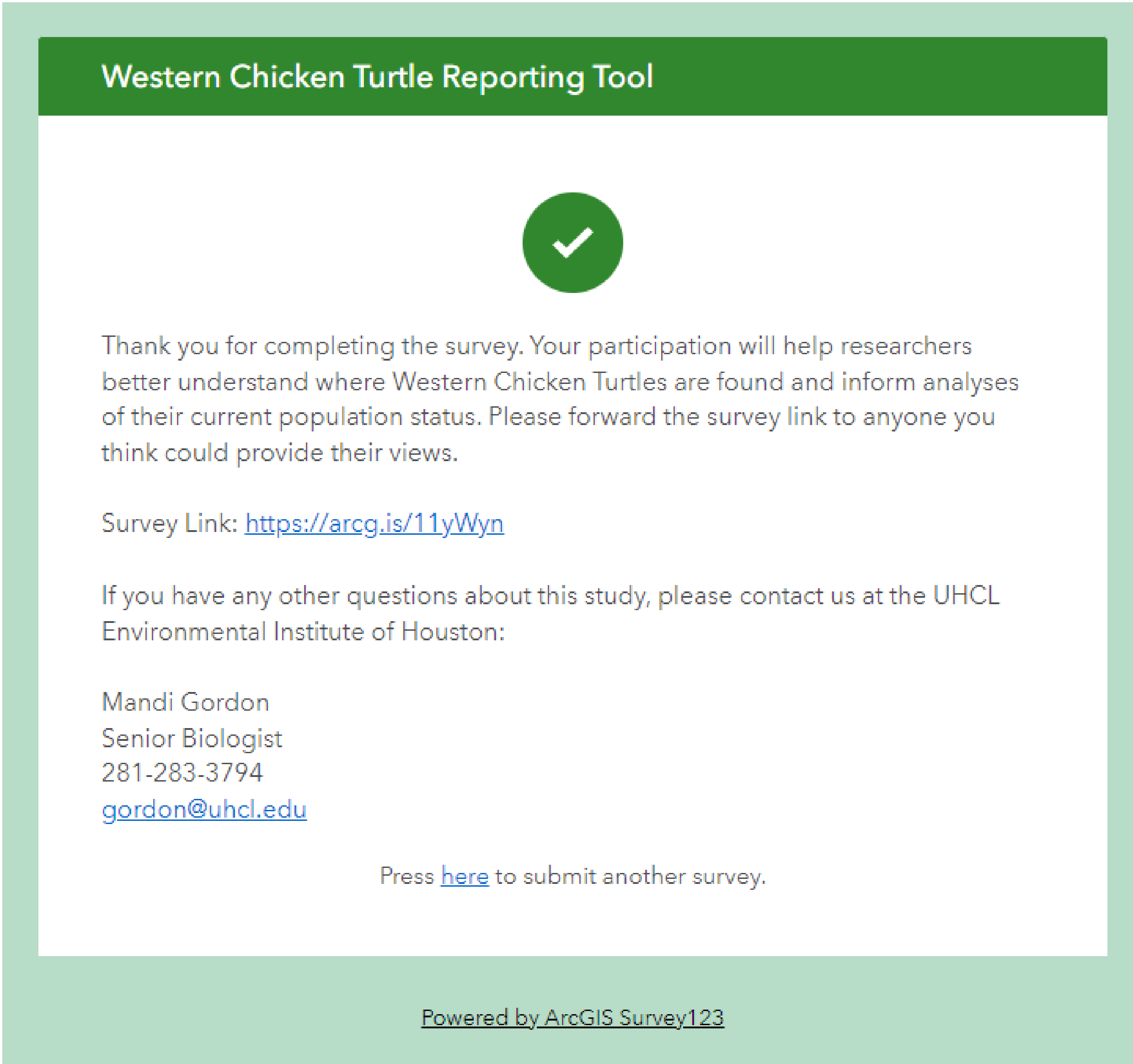
Back

Submit

Page 4 of 4

Powered by ArcGIS Survey123

Appendix Figure C.10 Page 3 of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This page allowed the respondent to provide contact information or remain anonymous. Responses to the reporting tool were not saved until respondents clicked "Submit" on this page.



Western Chicken Turtle Reporting Tool

Thank you for completing the survey. Your participation will help researchers better understand where Western Chicken Turtles are found and inform analyses of their current population status. Please forward the survey link to anyone you think could provide their views.

Survey Link: https://arcg.is/11yWyn

If you have any other questions about this study, please contact us at the UHCL Environmental Institute of Houston:

Mandi Gordon
Senior Biologist
281-283-3794
gordon@uhcl.edu

Press here to submit another survey.

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Appendix Figure C.11 Final page of the Online Reporting Tool developed for reports of Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas. This page thanked the respondent for completing the report and provided contact information for the project Co-PI if follow up information was needed. The respondent was also able to begin a new report from this page if multiple reports were necessary.

Appendix D – WCT Protocol Comparison Rubric

ELECTRONIC SUPPLEMENT

File Name: WCT Protocol Comparison Rubric_Final Report_Appendix D.xlsx

Description: Excel spreadsheet containing a blank copy of the protocol comparison rubric used for all methods used in this study. Includes descriptions, considerations, assumptions, and the scales used for each category and sub-category. Short descriptions for each scale value for each sub-category that can be used to score each method.

Appendix E – GIS Layers Used for Species Distribution Models

ELECTRONIC SUPPLEMENT

File Name: WCT SDM Layers_Final Report_Appendix E.mpkx

Description: Spatial datasets used for MaxEnt species distribution models (SDM). Includes occurrences used in the historic, current, and detection SDM, clipped data for environmental co-variates and MaxEnt ASCII layers for the historic, current, and detection SDM averaged outputs (deirochelys_avg_historic.asc, deirochelys_avg_current.asc, deirochelys_avg_detection.asc, respectfully).

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Appendix F – Taxonomic Groups Observed for Each Method

This appendix includes tables of all taxonomic groups observed for each protocol. Species are listed in order of highest to lowest relative abundance for each major group. Appendix Table F.1 details herpetofaunal species (verified using Bonnet et al., 2017) while Appendix Table F.2 details fish species (verified using Page et al., 2013).

Appendix Table F.1 List of herpetofaunal species observed for all protocols. Number of observations listed by survey method (BAVS = binocular assisted visual surveys; CSS = canid scent survey; RS = road survey; WS = walking survey; Hoop Trap, Drone = drone surveys [includes observations from both platform types]). Overall relative abundance ("Rel. Abund."; calculated across all taxonomic groups), total number of individuals observed (*N*), and count of taxonomic groups observed (S) also reported. Species are listed in order of highest to lowest relative abundance for each major group. Scientific names were verified using Bonnett et al. (2017).

Major Group	Lowest Taxonomic Level	Species	Common Name	BAVS	CSS	RS	WS	Hoop Trap	Drone M2	Drone P4	Total	Rel. Abund.
Amphibians	Species	*Lithobates catesbeianus*	American Bullfrog	397		30	18	8	11	13	477	0.041
Amphibians	Order	Anura	Unknown frog/toad	58			82		12	8	160	0.014
Amphibians	Species	*Lithobates sphenocephalus*	Southern Leopard Frog	49			93				142	0.012
Amphibians	Species	*Acris blanchardi*	Blanchard's Cricket Frog	2			45				47	0.004
Amphibians	Genus	*Acris sp.*	Cricket Frog	26			4				30	0.003
Amphibians	Subfamily	Acridinae	Cricket & Chorus Frogs, Spring Peepers	4			11				15	0.001
Amphibians	Species	*Hyla cinereus*	Green Treefrog	5			6				11	0.001
Amphibians	Species	*Incilius nebulifer*	Gulf Coast Toad				7				7	0.001
Amphibians	Family	Bufonidae	Unknown toad	1			6				7	0.001
Amphibians	Species	*Anaxyrus terrestris*	Southern Toad	1			5				6	0.001
Amphibians	Species	*Anaxyrus woodhousii*	Woodhouse's Toad				5				5	< 0.001
Amphibians	Species	*Notopthalmus viridescens*	Eastern Newt				2				2	< 0.001
Amphibians	Species	*Anaxyrus americanus*	American Toad				1				1	< 0.001
Amphibians	Genus	*Lithobates sp.*	American Water Frog	1							1	< 0.001
Amphibians	Species	*Acris crepitans*	Eastern Cricket Frog				1				1	< 0.001
Amphibians	Species	*Gastrophryne carolinensis*	Eastern Narrow-mouthed Toad				1				1	< 0.001
Amphibians	Species	*Anaxyrus fowleri*	Fowler's Toad				1				1	< 0.001
Amphibians	Species	*Gastrophryne olivacea*	Western Narrow-mouthed Toad	1							1	< 0.001
Amphibians	Species	*Pseudacris clarkii*	Spotted Chorus Frog				1				1	< 0.001
Amphibians	Class	Amphibia	Unknown tadpole	1							1	< 0.001
Reptiles	Species	*Alligator mississippiensis*	American Alligator	184		16	5	8	13	8	234	0.020
Reptiles	Genus	*Nerodia sp.*	North American Watersnake	36							36	0.003
Reptiles	Suborder	Serpentes	Unknown snake	12		3	7		3	3	28	0.002
Reptiles	Species	*Nerodia rhombifer*	Diamond-backed Watersnake	21			2	2	1		26	0.002
Reptiles	Subspecies	*Nerodia fasciata confluens*	Broad-banded Watersnake	16			5		2		23	0.002
Reptiles	Species	*Thamnophis proximus*	Western Ribbonsnake	10			8				18	0.002
Reptiles	Family	Scincidae	Unknown skink				12				12	0.001
Reptiles	Species	*Nerodia erythrogaster*	Plain-bellied Watersnake	6			4				10	0.001
Reptiles	Species	*Agkistrodon piscivorus*	Northern Cottonmouth	3			6				9	0.001
Reptiles	Species	*Anolis carolinensis*	Green Anole	7			1				8	0.001
Reptiles	Species	*Scincella lateralis*	Little Brown Skink				8				8	0.001
Reptiles	Species	*Pantherophis obsoletus*	Western Ratsnake	1		2	2				5	< 0.001
Reptiles	Species	*Eumeces fasciatus*	Common Five-lined skink	1			3				4	< 0.001
Reptiles	Genus	*Anolis sp.*	Anole lizard				3				3	< 0.001
Reptiles	Species	*Agkistrodon contortrix*	Eastern Copperhead				2				2	< 0.001

Appendix Table F.1 List of herpetofaunal species observed for all protocols. Number of observations listed by survey method (BAVS = binocular assisted visual surveys; CSS = canid scent survey; RS = road survey; WS = walking survey; Hoop Trap, Drone = drone surveys [includes observations from both platform types]). Overall relative abundance ("Rel. Abund."; calculated across all taxonomic groups), total number of individuals observed (*N*), and count of taxonomic groups observed (S) also reported. Species are listed in order of highest to lowest relative abundance for each major group. Scientific names were verified using Bonnett et al. (2017).

Major Group	Lowest Taxonomic Level	Species	Common Name	BAVS	CSS	RS	WS	Hoop Trap	Drone M2	Drone P4	Total	Rel. Abund.
Reptiles	Species	*Storeria dekayi*	Dekay's Brownsnake				2				2	< 0.001
Reptiles	Species	*Regina grahamii*	Graham's Crayfish Snake	1			1				2	< 0.001
Reptiles	Infraorder	Alethinophidia	North American Watersnake/Cottonmouth	2							2	< 0.001
Reptiles	Genus	*Thamnophis sp.*	North American Gartersnake	2							2	< 0.001
Reptiles	Order	Squamata	Unknown lizard			1	1				2	< 0.001
Reptiles	Species	*Micrurus tener*	Texas Coralsnake	1							1	< 0.001
Reptiles	Species	*Heterodon platirhinos*	Eastern Hog-nosed Snake			1					1	< 0.001
Reptiles	Species	*Thamnophis sirtalis*	Common Gartersnake	1							1	< 0.001
Reptiles	Species	*Coluber constrictor*	North American Racer				1				1	< 0.001
Reptiles	Species	*Diadophis punctatus*	Ringed-necked Snake				1				1	< 0.001
Reptiles	Species	*Opheodrys aestivus*	Rough Greensnake				1				1	< 0.001
Reptiles	Species	*Aspidoscelis sexlineata*	Six-lined Racerunner			1					1	< 0.001
Turtles	Subspecies	*Trachemys scripta elegans*	Red-eared Slider	2578	5	145	47	110	262	119	3266	0.280
Turtles	Suborder	Cryptodira	Unknown turtle	1996	2	117	37		558	441	3151	0.270
Turtles	Genus	*Trachemys sp.*	Slider Turtle	475		23	13		1054	1314	2879	0.247
Turtles	Species	*Trachemys scripta*	Pond Slider	475		8	16	2			501	0.043
Turtles	Subfamily	*Deirochelyinae*	Unknown Pond Turtle	76		4	3			4	87	0.007
Turtles	Genus	*Apalone sp.*	North American Softshell	21		1	2		22	13	59	0.005
Turtles	Species	*Chelydra serpentina*	Snapping turtle	8		1	4	15	6	7	41	0.004
Turtles	Species	*Sternotherus odoratus*	Eastern Musk Turtle	27		3	1	4			35	0.003
Turtles	Species	*Apalone spinifera*	Spiny Softshell	10		4		1	3	10	28	0.002
Turtles	Subspecies	*Deirochelys reticularia miaria*	Western Chicken Turtle	14	2	2		3	5	1	27	0.002
Turtles	Genus	*Graptemys sp.*	Map Turtle	22					3		25	0.002
Turtles	Genus	*Pseudemys sp.*	Cooter Turtle	17		3					20	0.002
Turtles	Species	*Kinosternon subrubrum hippocrepis*	Mississippi Mud Turtle					20			20	0.002
Turtles	Species	*Pseudemys concinna*	River Cooter	15			2	1			18	0.002
Turtles	Species	*Kinosternon subrubrum*	Eastern Mud Turtle	4	4		7		1		16	0.001
Turtles	Species	*Terrapene carolina*	Eastern Box Turtle		12		2				14	0.001
Turtles	Subspecies	*Graptemys pseudogeographica kohnii*	Mississippi Map Turtle	12							12	0.001
Turtles	Species	*Terrapene spp.*	American Box Turtle		5		1				6	0.001
Turtles	Subfamily	Kinosternidae	Mud/Musk Turtles							5	5	< 0.001
Turtles	Genus	*Sternotherus sp.*	Musk Turtle	2					1	1	4	< 0.001
Turtles	Genus	*Kinosternon sp.*	American Mud Turtle			3					3	< 0.001
Turtles	Species	*Chrysemys picta*	Painted Turtle	1			1				2	< 0.001
Turtles	Species	*Sternotherus carinatus*	Razor-backed Musk Turtle	2							2	< 0.001
Turtles	Species	*Apalone mutica*	Smooth softshell turtle						1		1	< 0.001
Turtles	Species	*Graptemys versa*	Texas Map Turtle	1							1	< 0.001
Unknown	Unknown	Unknown	Unknown	52	22	6	3	1		1	85	0.007
			Total Observed (*N*)	6,658	52	374	503	175	1,958	1,948	11,668	--
			Number of Taxonomic Levels Observed (S)	48	7	20	51	12	17	15	73	--

Appendix Table F.2 List of fish species observed during hoop trap surveys. Overall relative abundance ("Rel. Abund."; calculated across all taxonomic groups), total number of individuals observed (*N*), and count of taxonomic groups observed (S) also reported. Species are listed in order of highest to lowest relative abundance for each major group. Scientific names were verified using Page et al. (2013).

Major Group	Lowest Taxonomic Level	Species	Common Name	Count	Rel. Abund.
Fish	Species	*Lepomis macrochirus*	Bluegill	56	0.206
Fish	Species	*Ameiurus melas*	Black bullhead	55	0.202
Fish	Species	*Lepomis gulosus*	Warmouth	53	0.195
Fish	Species	*Ameiurus natalis*	Yellow bullhead	19	0.070
Fish	Species	*Lepomis cyanellus*	Green sunfish	19	0.070
Fish	Species	*Micropterus salmoides*	Largemouth bass	18	0.066
Fish	Family	*Cambaridae*	Unknown crawfish	16	0.059
Fish	Species	*Pomoxis annularis*	White crappie	12	0.044
Fish	Family	*Centrarchidae*	Unknown sunfish	10	0.037
Fish	Species	*Ictalurus punctatus*	Channel catfish	4	0.015
Fish	Order	*Siluriformes*	Unknown catfish	2	0.007
Fish	Species	*Pylodictis olivaris*	Flathead catfish	2	0.007
Fish	Superclass	Actinopterygii	Unknown ray-finned fish	1	0.004
Fish	Genus	*Pomoxis*	Unknown crappie	1	0.004
Fish	Species	*Erimyzon oblongus*	Creek chubsucker	1	0.004
Fish	Species	*Amia calva*	Bowfin	1	0.004
Fish	Species	*Lepisosteus oculatus*	Spotted gar	1	0.004
Fish	Species	*Atractosteus spatula*	Alligator gar	1	0.004
			Total Observed (*N*)	272	--
			Number of Taxonomic Levels Observed (S)	18	--

Literature Cited for Appendix F

Bonnett, 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.

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Appendix G – Occupancy Status by County

This appendix includes a full list of the 79 counties recognized as within the historic range for Western Chicken Turtles (WCT, *Deirochelys reticularia miaria*) in east Texas. Sources for historical occupancy status, date of last record, and counties with confirmed or potential detections in the current study are also included. Table also includes updated records for counties with historic occurrences (after the year 2000) for use in randomized site selection or species distribution models. Historic occurrences were extracted from the Global Biodiversity Information Facility (GBIF, 2022), iNaturalist (iNaturalist, 2020), VertNet (VertNet, 2020), literature (Adams and Saenz, 2011; Ryberg et al., 2017; Franklin et al., 2019; Bowers et al., 2021, 2022), or from personal communications with professionals and experts in the field. “Not detected” is noted in the Detection Type column when WCT were not detected in the current study.

Appendix Table G.1 Occupancy status for the 79 counties within the historic range of the Western Chicken Turtle (WCT; Deirochelys reticularia miaria) in east Texas. Status based on historic range from Dixon (2013) and USFWS (2016). Last record includes year of last reported record based on results of the current study or previous studies. “Detection type” refers to the type of detection made in the current study (Confirmed = confirmed detection using field methods or photo-verified results of the online reporting tool; Potential = potential detection based on results of eDNA protocol(s); Not detected = county was sampled in the current study but did not result in detections of WCT using any protocol applied). Occupancy source includes a list of citations for all sources of occupancy data.

County	Historical Status	Last Record	Detection Type	Occupancy Source
Anderson	Occupied	2021	Potential	Dixon, 2013; USFWS, 2016; current study
Angelina	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Austin	Occupied	2022	Confirmed	Dixon, 2013; USFWS, 2016; current study
Bowie	Occupied	Unknown	--	USFWS, 2016
Brazoria	Occupied	2021	Confirmed	Dixon, 2013; USFWS, 2016; GBIF, 2022; current study
Brazos	Occupied	Since 2016	--	Dixon, 2013; USFWS, 2016; iNaturalist, 2020; VertNet 2020
Burleson	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Camp	Occupied	Unknown	--	USFWS, 2016
Cass	Occupied	Since 2020	--	USFWS, 2016; R. Speight (*personal communication*)
Chambers	Occupied	2022	Confirmed	Dixon, 2013; USFWS, 2016; iNaturalist, 2020; GBIF, 2022; current study
Cherokee	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Collin	Occupied	Unknown	--	USFWS, 2016
Colorado	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Dallas	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Delta	Occupied	2021	Potential	Dixon, 2013; USFWS, 2016; current study
Denton	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Ellis	Occupied	Unknown	--	USFWS, 2016
Falls	Occupied	Since 2015	--	Dixon, 2013; USFWS, 2016; Ryberg et al., 2017
Fannin	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Fayette	Occupied	Unknown	--	USFWS, 2016
Fort Bend	Occupied	Since 2019	Not detected	Dixon, 2013; USFWS, 2016; Franklin et al., 2019; iNaturalist, 2020

Appendix Table G.1 Occupancy status for the 79 counties within the historic range of the Western Chicken Turtle (WCT; Deirochelys reticularia miaria) in east Texas. Status based on historic range from Dixon (2013) and USFWS (2016). Last record includes year of last reported record based on results of the current study or previous studies. "Detection type" refers to the type of detection made in the current study (Confirmed = confirmed detection using field methods or photo-verified results of the online reporting tool; Potential = potential detection based on results of eDNA protocol(s); Not detected = county was sampled in the current study but did not result in detections of WCT using any protocol applied). Occupancy source includes a list of citations for all sources of occupancy data.

County	Historical Status	Last Record	Detection Type	Occupancy Source
Franklin	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Freestone	Occupied	2021	Potential	Dixon, 2013; USFWS, 2016; current study
Galveston	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Grayson	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Gregg	Occupied	Unknown	--	USFWS, 2016
Grimes	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Hardin	Occupied	Since 2017	Not detected	Dixon, 2013; USFWS, 2016; iNaturalist, 2020
Harris	Occupied	2021	Confirmed	Dixon, 2013; USFWS, 2016; Ryberg et al., 2017; Bowers et al., 2021; GBIF, 2022; current study
Harrison	Occupied	Since 2019	Not detected	Dixon, 2013; USFWS, 2016; iNaturalist, 2020; C. Roelke (*personal communication*)
Henderson	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Hill	Occupied	Since 2008	--	Dixon, 2013; USFWS, 2016
Hopkins	Occupied	2022	Confirmed	Dixon, 2013; USFWS, 2016; current study
Houston	Occupied	Unknown	--	USFWS, 2016
Hunt	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Jasper	Occupied	2021	Confirmed	Dixon, 2013; USFWS, 2016; current study
Jefferson	Occupied	2022	Confirmed	Dixon, 2013; USFWS, 2016; iNaturalist, 2020; GBIF, 2022; current study
Kaufman	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Lamar	Occupied	Unknown	--	USFWS, 2016
Lavaca	Occupied	Unknown	--	Dixon, 2013
Lee	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Leon	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Liberty	Occupied	Since 2013	--	Dixon, 2013; USFWS, 2016; iNaturalist, 2020; VertNet, 2020
Limestone	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Madison	Occupied	Unknown	--	USFWS, 2016
Marion	Occupied	Unknown	--	USFWS, 2016
McLennan	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Milam	Occupied	Since 2010	Not detected	USFWS, 2016; iNaturalist, 2020
Montgomery	Occupied	Since 2008	--	Dixon, 2013; USFWS, 2016
Morris	Occupied	Unknown	--	USFWS, 2016
Nacogdoches	Occupied	2022	Confirmed	Adams and Saenz, 2011; Dixon, 2013; USFWS, 2016; iNaturalist, 2020; Bowers et al., 2022a; current study

Appendix Table G.1 Occupancy status for the 79 counties within the historic range of the Western Chicken Turtle (WCT; Deirochelys reticularia miaria) in east Texas. Status based on historic range from Dixon (2013) and USFWS (2016). Last record includes year of last reported record based on results of the current study or previous studies. "Detection type" refers to the type of detection made in the current study (Confirmed = confirmed detection using field methods or photo-verified results of the online reporting tool; Potential = potential detection based on results of eDNA protocol(s); Not detected = county was sampled in the current study but did not result in detections of WCT using any protocol applied). Occupancy source includes a list of citations for all sources of occupancy data.

County	Historical Status	Last Record	Detection Type	Occupancy Source
Navarro	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Newton	Occupied	Unknown	--	USFWS, 2016
Orange	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Panola	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Polk	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Rains	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Red River	Occupied	Unknown	--	USFWS, 2016
Robertson	Occupied	Unknown	Not detected	USFWS, 2016; iNaturalist, 2020
Rockwall	Occupied	2021	Potential	USFWS, 2016; current study
Rusk	Occupied	Unknown	--	USFWS, 2016
Sabine	Occupied	Unknown	--	USFWS, 2016
San Augustine	Occupied	Unknown	--	USFWS, 2016
San Jacinto	Occupied	Unknown	Not detected	USFWS, 2016
Shelby	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Smith	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Tarrant	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Titus	Occupied	Unknown	--	USFWS, 2016
Trinity	Occupied	Unknown	Not detected	USFWS, 2016
Tyler	Occupied	Unknown	Not detected	Dixon, 2013; USFWS, 2016
Upshur	Occupied	2022	Confirmed	USFWS, 2016; current study
Van Zandt	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Walker	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Waller	Occupied	2022	Confirmed	Dixon, 2013; USFWS, 2016; Ryberg et al., 2017; iNaturalist, 2020; VertNet, 2020; Bowers et al., 2021, 2022a; GBIF, 2022; current study
Washington	Occupied	Unknown	--	USFWS, 2016
Wharton	Occupied	Unknown	Not detected	Dixon, 2013; GBIF, 2022
Williamson	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Wise	Occupied	Unknown	--	Dixon, 2013; USFWS, 2016
Wood	Occupied	Unknown	Not detected	USFWS, 2016

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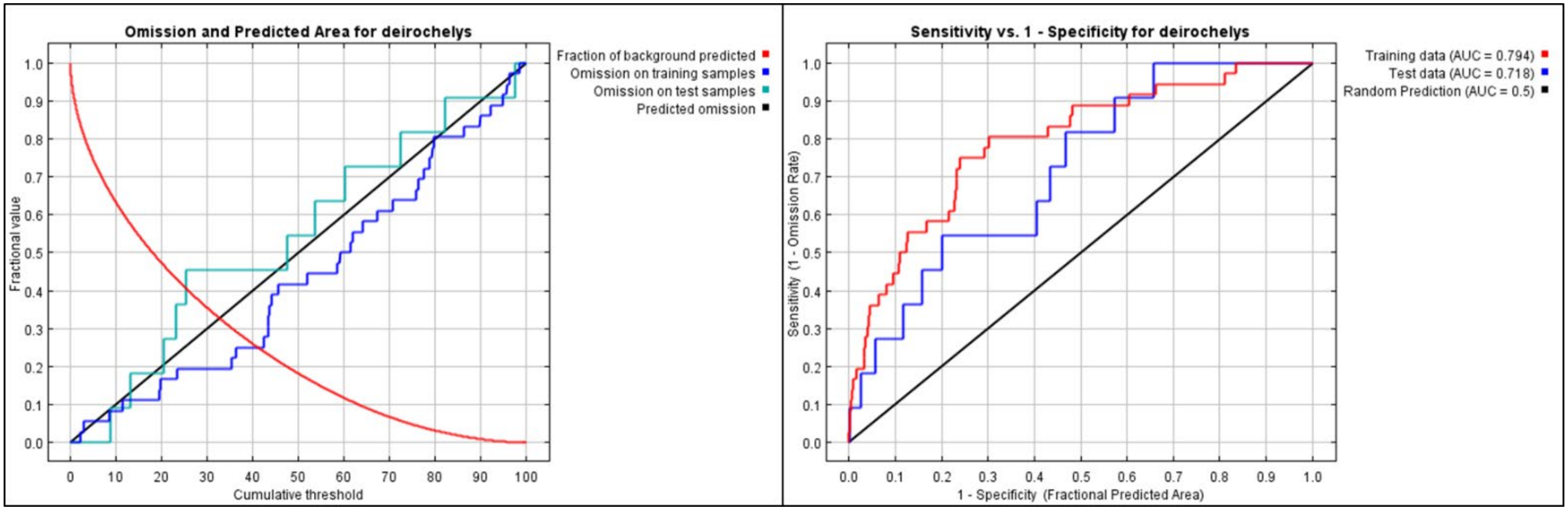
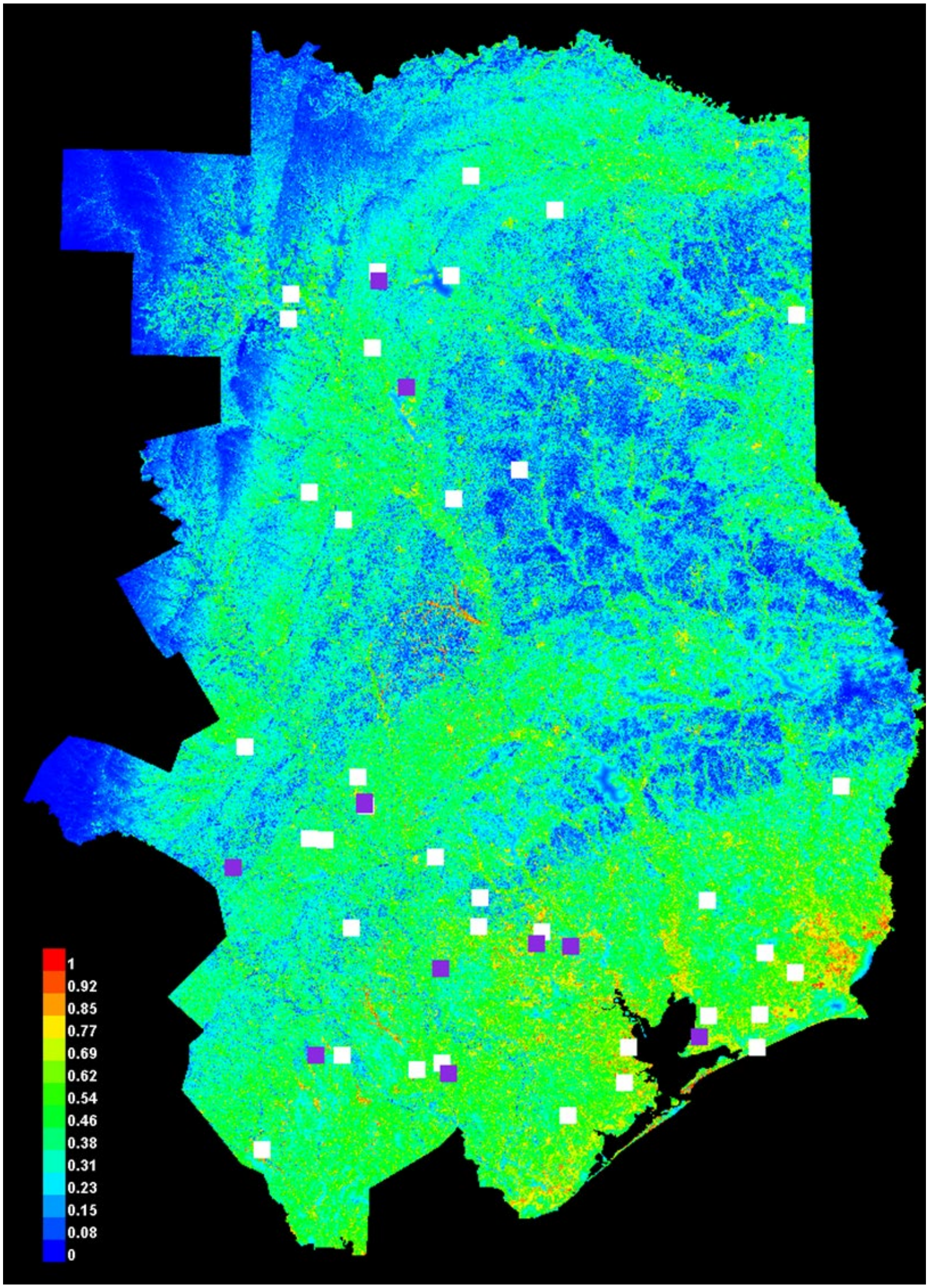
Appendix H – Historic Species Distribution Model Excluding Raw Land Cover and Road Density

This appendix contains the results of an additional MaxEnt species distribution model (SDM) run using historic WCT occurrences, but excluding raw land cover and road density co-variates as a “quick fix” in an attempt to remove urban sampling bias. The same methods as those described previously in this report were followed for data selection, compilation, and harmonization. The same MaxEnt settings were used as described previously except only one replicate was run and test data were set to 25%. Model outputs include statistical plots of model performance and fit, the predicted distribution map, and percent contribution and permutation importance of the environmental co-variates.



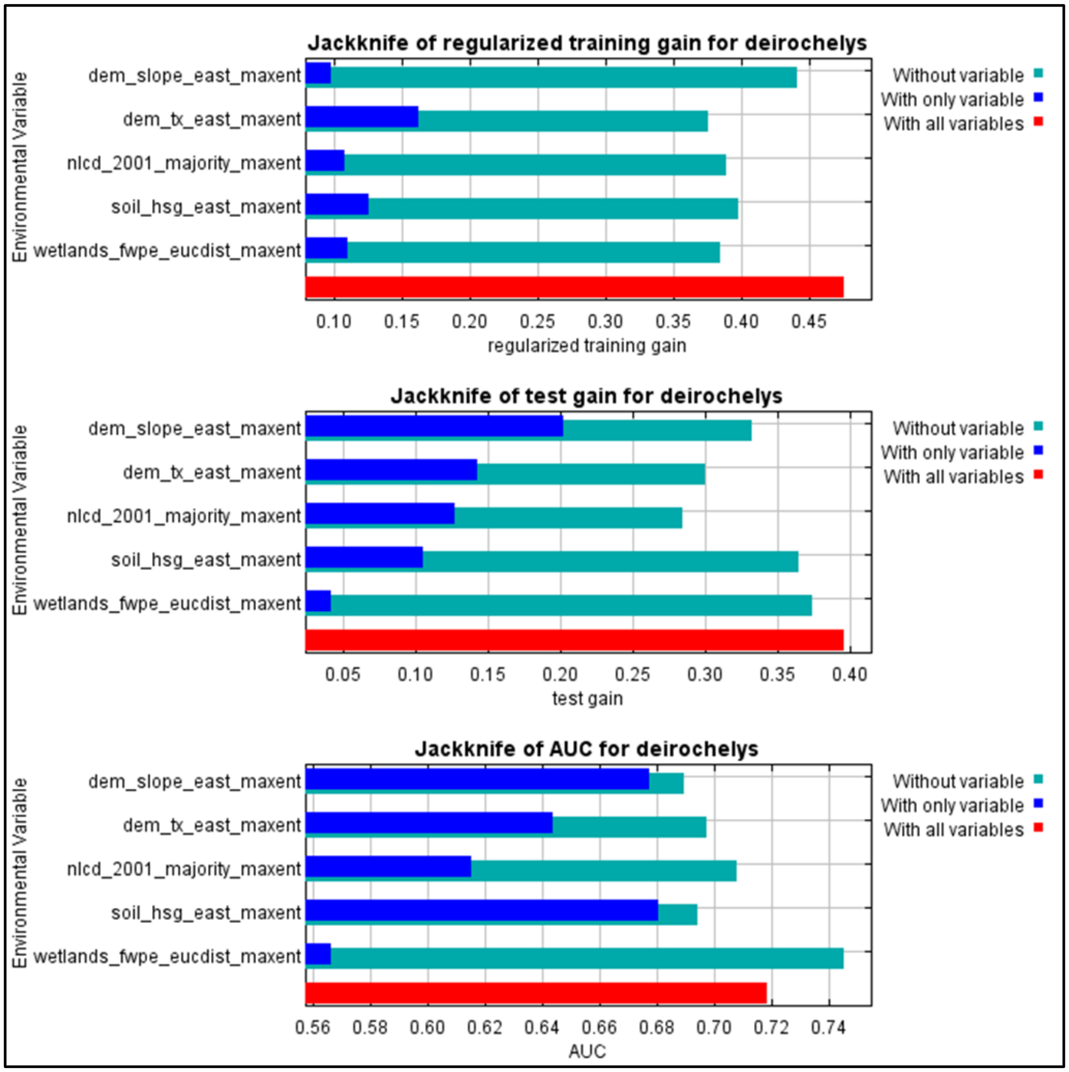
Appendix Figure H.1 Statistical plots for average omission and predicted area (left) and model sensitivity and specificity (right) of historic species distribution model (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas, excluding raw land cover class and road density. For omission and predicted area plot, better model performance is indicated by the training and test omission rates (dark and light blue lines, respectfully) being close to the predicted rate (black line). For model sensitivity and specificity, better fit is indicated by training and testing of the receiver operating characteristic (ROC) curve (red and blue lines, respectfully) being above the random prediction line (black line) and area under the curve (AUC) values > 0.75.



Appendix Figure H.2 Historic species distribution model (SDM) output from MaxEnt (includes occurrences from 2000 and earlier, $n = 47$) for Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) in east Texas, excluding raw land cover class and road density. Geographic extent is limited to counties within the species historic range + Jackson County. Environmental co-variate input layers included: elevation and slope (USGS, 2013), majority land cover (USGS, 2001), distance between freshwater emergent and ponded wetland types (USFWS, 2022), and hydrologic soil group (USDA, 2021). Habitat suitability is interpreted from probability of presence with more suitable habitat indicated by warmer colors (red) and less suitable habitat by cooler colors (blue). White dots are locations of occurrences used in model training; purple dots are occurrences used in model testing.

Appendix Table H.1 Percent contribution and permutation importance of each environmental co-variate used in historic species distribution model (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in east Texas, excluding raw land cover class and road density. Top three contributing and importance factors in italics.

Environmental co-variate	Contribution	Importance
Distance between wetlands	*22.1*	*21.8*
Elevation	19.3	*39.9*
Land cover class - majority	*19.4*	16.3
Slope	17.6	*17.2*
Soil class	*21.6*	4.8



Appendix Figure H.3 Jackknife test results of regularized training gain (top), test gain (middle), and area under the curve (AUC; bottom) for the historic species distribution model (SDM) for Western Chicken Turtles (WCT; *Deirochelys reticularia miaria*) in Texas, excluding raw land cover class and road density. Jackknife results show relative importance of environmental co-variates used as inputs with red bars representing models with all co-variates included, dark blue bars representing models with one co-variate used in isolation, and light blue bars representing models with one co-variate omitted. Jackknife of regularized training gain showed the model was best informed by elevation. Jackknife of test gain was more variable with slope causing the largest increase but majority land cover class causing the largest decrease. Jackknife of AUC was also different in that the co-variate containing the most information by itself was soil class (largest increase), while slope contained marginally more information not present in the other co-variates (largest decrease).

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Appendix I – Full List of Detectability Model

This appendix contains a full list of the detectability models, predictability co-variate type, specific model co-variates, Akaike Information Criteria, Akaike difference, and Akaike weight for all models by protocol type. The top three models for each protocol were assessed for goodness-of-fit using Pearson's X^2 Goodness of Fit test and the resulting detection probability (ρ) for the best-fit model was included in protocol comparison rubric calculations.

Appendix Table I.1 Detectability models tested for all protocols. Table includes model name, predictability co-variate type (ρ-type), model co-variates, Akaike Information Criteria (AIC), Akaike difference (ΔAIC), and Akaike weight (W_{AIC}). Model outputs are grouped by protocol and listed from lowest to highest AIC. "†" indicates best-fit model used in protocol comparison rubric.

Model Name	ρ-type	Model co-variates	AIC	ΔAIC	W_{AIC}
Environmental DNA - Ambient water with 0.45µm filter (n sites = 4)					
fm0†	time	Null	43.56	0.00	0.6392
fm3	time	Ψ (habitat)	45.56	2.00	0.2351
fm2	time	Ψ (wetland)	47.56	4.00	0.8651
fm5	time	Ψ (wetland+habitat)	49.56	6.00	0.0318
fm1.t	time	ρ (event)	53.36	9.80	0.0048
fm10.t	time	ρ (event), Ψ (habitat)	55.36	11.80	0.0018
fm9.t	time	ρ (event), Ψ (wetland)	57.36	13.80	0.0006
fm12.t	time	ρ (event), Ψ (wetland+habitat)	59.36	15.80	0.0002
Environmental DNA - Ambient water with 3.00µm filter (in-season events only; n sites = 10)					
fm0	time	Null	105.44	0.00	0.2752
fm2†	time	Ψ (wetland)	105.99	0.55	0.2092
fm3	time	Ψ (habitat)	106.24	0.80	0.1847
fm4	time	Ψ (criteria)	106.71	1.27	0.1455
fm5	time	Ψ (wetland+habitat)	107.98	2.55	0.0770
fm7	time	Ψ (habitat+criteria)	108.58	3.15	0.0571
fm6	time	Ψ (wetland+criteria)	109.89	4.45	0.0297
fm8	time	Ψ (wetland+habitat+criteria)	111.98	6.55	0.0104
fm1.t	time	ρ (event)	114.37	8.94	0.0032
fm9.t	time	ρ (event), Ψ (wetland)	114.82	9.39	0.0025
fm10.t	time	ρ (event), Ψ (habitat)	114.89	9.45	0.0024
fm11.t	time	ρ (event), Ψ (criteria)	116.27	10.83	0.0012
fm12.t	time	ρ (event), Ψ (wetland+habitat)	116.82	11.39	0.0009
fm14.t	time	ρ (event), Ψ (habitat+criteria)	117.83	12.39	0.0006
fm13.t	time	ρ (event), Ψ (wetland+criteria)	118.82	13.39	0.0003
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	120.82	15.39	0.0001
Environmental DNA - Ambient water with 3.00µm filter (out-of-season events only; n sites = 4)					
fm0	time	Null	69.19	0.00	0.3842
fm4	time	Ψ (criteria)	71.19	2.00	0.1414
fm3	time	Ψ (habitat)	71.19	2.00	0.1413
fm1.t	time	ρ (event)	72.18	2.98	0.0865
fm7	time	Ψ (habitat+criteria)	73.19	4.00	0.0520
fm2	time	Ψ (wetland)	73.19	4.00	0.0520
fm10.t	time	ρ (event), Ψ (habitat)	74.18	4.98	0.0318
fm11.t	time	ρ (event), Ψ (criteria)	74.18	4.98	0.0318
fm5	time	Ψ (wetland+habitat)	75.19	6.00	0.0191
fm6	time	Ψ (wetland+criteria)	75.19	6.00	0.0191
fm9.t	time	ρ (event), Ψ (wetland)	76.18	6.98	0.0117
fm14.t	time	ρ (event), Ψ (habitat+criteria)	76.18	6.98	0.0117
fm8	time	Ψ (wetland+habitat+criteria)	77.19	8.00	0.0070
fm12.t	time	ρ (event), Ψ (wetland+habitat)	78.18	8.98	0.0043

Appendix Table I.1 Detectability models tested for all protocols. Table includes model name, predictability co-variate type (ρ-type), model co-variates, Akaike Information Criteria (AIC), Akaike difference (ΔAIC), and Akaike weight (W_{AIC}). Model outputs are grouped by protocol and listed from lowest to highest AIC. "†" indicates best-fit model used in protocol comparison rubric.

Model Name	ρ-type	Model co-variates	AIC	ΔAIC	W_{AIC}
fm13.t	time	ρ (event), Ψ (wetland+criteria)	78.18	8.98	0.0043
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	80.18	10.98	0.0016
Environmental DNA - Resuspended sediment with 0.45μm filter (n sites = 4)					
fm0[a]	time	Null	31.36	--	--
fm2[†]	time	Ψ (wetland)	32.88	0.00	0.5576
fm3	time	Ψ (habitat)	33.36	0.48	0.4379
fm5[a]	time	Ψ (wetland+habitat)	34.88	--	--
fm1.t	time	ρ (event)	43.99	11.12	0.0022
fm9.t	time	ρ (event), Ψ (wetland)	45.28	12.40	0.0011
fm10.t	time	ρ (event), Ψ (habitat)	45.99	13.12	0.0008
fm12.t	time	ρ (event), Ψ (wetland+habitat)	47.28	14.40	0.0004
Environmental DNA - Resuspended sediment with 3.0μm filter (in-season events only; n sites = 10)					
fm0	time	Null	97.82	0.00	0.2788
fm2	time	Ψ (wetland)	98.53	0.71	0.7959
fm6[†]	time	Ψ (wetland+criteria)	99.34	1.52	0.1306
fm5	time	Ψ (wetland+habitat)	99.53	1.71	0.1188
fm3	time	Ψ (habitat)	99.75	1.93	0.1062
fm4	time	Ψ (criteria)	100.57	2.75	0.0705
fm8	time	Ψ (wetland+habitat+criteria)	100.72	2.90	0.0654
fm7	time	Ψ (habitat+criteria)	102.55	4.73	0.0263
fm1.t	time	ρ (event)	107.42	9.60	0.0023
fm9.t	time	ρ (event), Ψ (wetland)	108.40	10.57	0.0014
fm12.t	time	ρ (event), Ψ (wetland+habitat)	109.29	11.47	0.0009
fm13.t	time	ρ (event), Ψ (wetland+criteria)	109.40	11.58	0.0009
fm10.t	time	ρ (event), Ψ (habitat)	109.41	11.58	0.0009
fm11.t	time	ρ (event), Ψ (criteria)	110.08	12.26	0.0006
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	110.52	12.70	0.0005
fm14.t	time	ρ (event), Ψ (habitat+criteria)	112.00	14.18	0.0002
Environmental DNA - Resuspended sediment with 3.0μm filter (out-of-season events only; n sites = 4)					
fm0	time	Null	70.31	0.00	0.4700
fm4	time	Ψ (criteria)	72.31	2.00	0.1700
fm3	time	Ψ (habitat)	72.31	2.00	0.1700
fm7	time	Ψ (habitat+criteria)	74.31	4.00	0.0640
fm2	time	Ψ (wetland)	74.31	4.00	0.0640
fm6	time	Ψ (wetland+criteria)	76.31	6.00	0.0230
fm5	time	Ψ (wetland+habitat)	76.31	6.00	0.0230
fm8	time	Ψ (wetland+habitat+criteria)	78.31	8.00	0.0086
fm1.t	time	ρ (event)	83.95	13.64	0.0005
fm10.t	time	ρ (event), Ψ (habitat)	85.95	15.64	0.0002
fm11.t	time	ρ (event), Ψ (criteria)	85.95	15.64	0.0002
fm9.t	time	ρ (event), Ψ (wetland)	87.95	17.64	0.0001
fm14.t	time	ρ (event), Ψ (habitat+criteria)	87.95	17.64	0.0001
fm12.t	time	ρ (event), Ψ (wetland+habitat)	89.95	19.64	< 0.0001
fm13.t	time	ρ (event), Ψ (wetland+criteria)	89.95	19.64	< 0.0001
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	91.95	21.64	< 0.0001
Environmentall DNA - Soil (n sites = 6)					
fm0	time	Null	21.18	0.00	0.6100
fm4 [a]	time	Ψ (criteria)	22.63	1.45	--
fm3[†]	time	Ψ (habitat)	23.18	2.00	0.2300
fm7 [a]	time	Ψ (habitat+criteria)	24.50	3.32	--

Appendix Table I.1 Detectability models tested for all protocols. Table includes model name, predictability co-variate type (ρ-type), model co-variates, Akaike Information Criteria (AIC), Akaike difference (ΔAIC), and Akaike weight (W_{AIC}). Model outputs are grouped by protocol and listed from lowest to highest AIC. "†" indicates best-fit model used in protocol comparison rubric.

Model Name	ρ-type	Model co-variates	AIC	ΔAIC	W_{AIC}
fm2	time	Ψ (wetland)	25.45	4.27	0.0730
fm5 [a]	time	Ψ (wetland+habitat)	25.77	4.59	--
fm6	time	Ψ (wetland+criteria)	27.45	6.27	0.0620
fm8	time	Ψ (wetland+habitat+criteria)	27.77	6.59	0.0230
fm1.t	time	ρ (event)	36.01	14.82	0.0004
fm11.t	time	ρ (event), Ψ (criteria)	37.00	15.81	0.0002
fm10.t	time	ρ (event), Ψ (habitat)	37.99	16.80	0.0001
fm14.t	time	ρ (event), Ψ (habitat+criteria)	38.63	17.45	0.0001
fm12.t	time	ρ (event), Ψ (wetland+habitat)	39.55	18.36	0.0001
fm9.t	time	ρ (event), Ψ (wetland)	39.58	18.40	0.0001
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	41.55	20.36	< 0.0001
fm13.t	time	ρ (event), Ψ (wetland+criteria)	41.58	20.40	< 0.0001
Binocular Assisted Visual Surveys (n sites = 10)					
fm3	time	Ψ (habitat)	30.87	0.00	0.6500
fm0	time	Null	34.15	3.28	0.1300
fm7	time	Ψ (habitat+criteria)	34.37	3.49	0.1100
fm5	time	Ψ (wetland+habitat)	35.81	4.94	0.0550
fm4	time	Ψ (criteria)	37.65	6.77	0.0220
fm2	time	Ψ (wetland)	38.72	7.84	0.0130
fm8	time	Ψ (wetland+habitat+criteria)	39.81	8.94	0.0075
fm10.t	time	ρ (event), Ψ (habitat)	41.18	10.31	0.0038
fm6	time	Ψ (wetland+criteria)	41.63	10.76	0.0030
fm11.t	time	ρ (event), Ψ (criteria)	44.45	13.57	0.0005
fm14.t	time	ρ (event), Ψ (habitat+criteria)	45.18	14.31	0.0005
fm1.t	time	ρ (event)	45.23	14.36	0.0002
fm12.t	time	ρ (event), Ψ (wetland+habitat)	47.18	16.31	0.0001
fm9.t	time	ρ (event), Ψ (wetland)	50.14	19.26	< 0.0001
fm15.t	time	ρ (event), Ψ (wetland+habitat+criteria)	51.18	20.31	< 0.0001
fm13.t	time	ρ (event), Ψ (wetland+criteria)	52.72	21.85	< 0.0001
fm10.e[†]	effort	ρ (effort), Ψ (habitat)	29.85	0.00	0.4215
fm3	effort	Ψ (habitat)	30.87	1.02	0.2528
fm1.e	effort	ρ (effort)	33.47	3.62	0.0692
fm14.e	effort	ρ (effort), Ψ (habitat+criteria)	33.51	3.66	0.0675
fm0	effort	Null	34.15	4.30	0.0491
fm7	effort	Ψ (habitat+criteria)	34.37	4.51	0.0441
fm12.e	effort	ρ (effort), Ψ (wetland+habitat)	35.31	5.46	0.0275
fm5	effort	Ψ (wetland+habitat)	35.81	5.96	0.0214
fm11.e	effort	ρ (effort), Ψ (criteria)	36.67	6.82	0.0139
fm9.e	effort	ρ (effort), Ψ (wetland)	37.45	7.59	0.0095
fm4	effort	Ψ (criteria)	37.65	7.79	0.0086
fm2	effort	Ψ (wetland)	38.72	8.86	0.0050
fm15.e	effort	ρ (effort), Ψ (wetland+habitat+criteria)	39.31	9.46	0.0037
fm8	effort	Ψ (wetland+habitat+criteria)	39.81	9.96	0.0029
fm13.e	effort	ρ (effort), Ψ (wetland+criteria)	40.40	10.55	0.0022
fm6	effort	Ψ (wetland+criteria)	41.63	11.78	0.0012
Canid scent surveys (n sites = 2)					
fm0[a]	time	Null	11.35	--	--
fm3[a]	time	Ψ (habitat)	12.03	--	--
fm4[a]	time	Ψ (criteria)	12.03	--	--
fm7	time	Ψ (habitat+criteria)	14.03	0.00	0.8670

Appendix Table I.1 Detectability models tested for all protocols. Table includes model name, predictability co-variate type (ρ-type), model co-variates, Akaike Information Criteria (AIC), Akaike difference (ΔAIC), and Akaike weight (W_{AIC}). Model outputs are grouped by protocol and listed from lowest to highest AIC. "†" indicates best-fit model used in protocol comparison rubric.

Model Name	ρ-type	Model co-variates	AIC	ΔAIC	W_{AIC}
fm10.t	time	ρ (event), Ψ (habitat)	20.00	5.97	0.0440
fm11.t	time	ρ (event), Ψ (criteria)	20.00	5.97	0.0440
fm1.t	time	ρ (event)	20.77	6.74	0.0300
fm14.t	time	ρ (event), Ψ (habitat+criteria)	22.00	7.97	0.0160
fm0[a]	effort	Null	11.35	--	--
fm3[a]	effort	Ψ (habitat)	12.03	--	--
fm4[a]	effort	Ψ (criteria)	12.03	--	--
fm1.e[a]	effort	ρ (effort)	12.08	--	--
fm10.e[a]	effort	ρ (effort), Ψ (habitat)	13.34	--	--
fm11.e[a]	effort	ρ (effort), Ψ (criteria)	13.34	--	--
fm7 [b]	effort	Ψ (habitat+criteria)	14.03	0.00	--
fm14.e[†,b]	effort	ρ (effort), Ψ (habitat+criteria)	15.33	1.30	--
Hoop trap surveys (n sites = 3)					
fm0[†]	time	Null	14.81	0.00	0.4100
fm3	time	Ψ (habitat)	15.00	0.18	0.3800
fm10.t	time	ρ (event), Ψ (habitat)	17.55	2.73	0.1100
fm1.t	time	ρ (event)	17.64	2.82	0.1000
Camera trap (n sites = 3)					
fm0[†]	time	Null	14.46	0.00	1.0000
fm3[a]	time	Ψ (habitat)	15.31	--	--
fm1.t	time	ρ (event)	39.82	25.35	< 0.0001
fm10.t	time	ρ (event), Ψ (habitat)	40.77	26.31	< 0.0001
Drone - Mavic 2 Video Imagery (n sites = 4)					
fm0	time	Null	18.70	0.00	0.5730
fm3[†]	time	Ψ (habitat)	20.03	1.32	0.2960
fm1.t	time	ρ (event)	22.50	3.79	0.0860
fm10.t	time	ρ (event), Ψ (habitat)	23.82	5.11	0.0440
fm1.e	effort	ρ (effort)	17.65	0.00	0.5300
fm0	effort	Null	18.70	1.06	0.3100
fm10.e[a]	effort	ρ (effort), Ψ (habitat)	19.59	1.94	--
fm3	effort	Ψ (habitat)	20.03	2.38	0.1600
Drone - Phantom 4 Multispectral Imagery (n sites = 4)					
fm0[a]	time	Null	11.35	--	--
fm3[a]	time	Ψ (habitat)	12.70	--	--
fm1.t	time	ρ (event)	22.50	0.00	0.6600
fm10.t[†]	time	ρ (event), Ψ (habitat)	23.82	1.32	0.3400

[a]Model did not converge and was not used in calculations for ΔAIC or W_{AIC}.
[b] Unable to compare models for W_{AIC} calculation due to convergence issues between models.