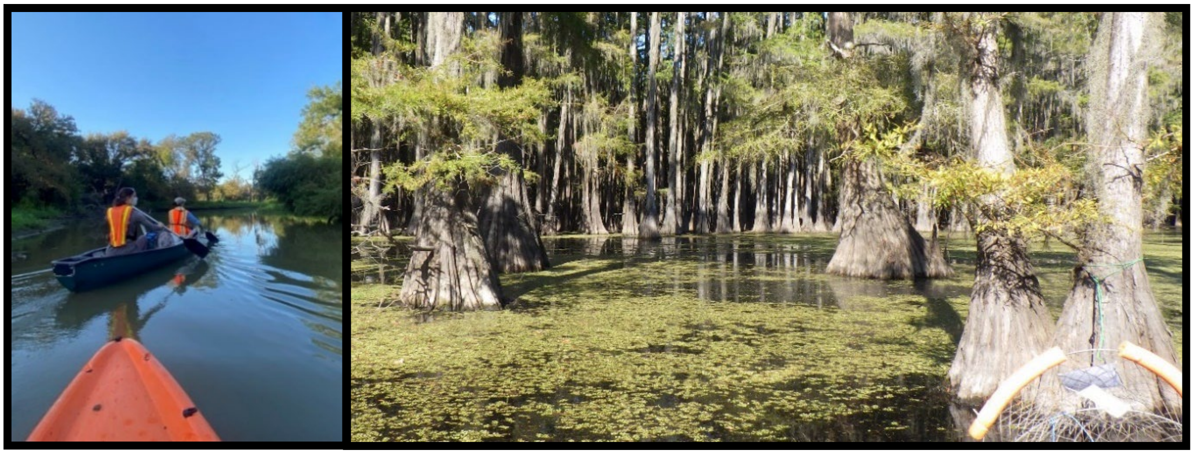
Baseline Study of Alligator Snapping Turtle (*Macrochelys temminckii*) Population Viability in Texas Watersheds

Final Report

EIH Final Report #EIH23-002
Final: 06 March 2023

Prepared by the Environmental Institute of Houston at the University of Houston – Clear Lake in cooperation with the Texas Comptroller of Public Accounts

Environmental Institute of Houston



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Prepared in cooperation with and for the Texas Comptroller of Public Accounts

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Recommended citation: Gordon, M, B. Giles, K. Garcia, J.W. Oakley, E. Munscher, J.J. Apodaca, A. Krohn, D.R. Bontrager, J. Kittle, M. Mokrech, T. Sankey, A. Tuggle, C. Franklin, V. Ricardez, C. Schalk, D. Saenz, D. Rosenbaum, C. Jones, J. Watson, T. Corbett, and G. Guillen. 2023. Baseline study of Alligator Snapping Turtle (*Macrochelys temminckii*) population viability in Texas watersheds, Final Report. (Report No. EIH23-002). Prepared for the Texas Comptroller of Public Accounts (Contract 20-6997BG). 107 pages.

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ACKNOWLEDGEMENTS

The success of this project is largely attributed to the extensive collaboration between private landowners, stakeholders, agency professionals, and other research institutions throughout east Texas. This has truly been a Herculean effort and the authors are grateful to all that participated.

We would like to thank Connor Adams and Dr. Johanna Delgado-Acevedo for their communication and coordination between existing surveys of ASTs. The volunteers of Texas Turtles and the Turtle Survival Alliance's North American Freshwater Turtle Research Group were integral in collaborative field efforts. We also thank Dr. Paul Crump with Texas Parks and Wildlife Department for his feedback in sample design development, site selection, and assistance with data sharing. Christopher Maldonado was also particularly helpful in answering questions related to permitting.

The Angelina-Neches River Authority, Brazos River Authority, Lower Neches Valley Authority (LNVA), Northeast Texas Municipal Water District, Tarrant Regional Water District, Trinity River Authority, and Water Monitoring Solutions, Ltd. aided with providing landowner contacts and spreading information about the project to their constituents. Bill Kirby was particularly helpful with compiling historic account information and providing housing during field surveys. Jason Watson and Terry Corbett were responsible for creation of the LNVA Mapping tool and integral in its upkeep and data sharing. Web Mangham and Kelly McKnight were especially helpful with access information and field surveys within the Trinity River basin. Laura Ashley Overdye also assisted with landowner contacts and coordination in Caddo Lake. The U.S. Army Corps of Engineers (especially Floyd Boyett and Brian Heffernan) provided boats and assisted with access near B.A. Steinhagen Lake and Lake Sam Rayburn. Jason Watson and Terry Corbett were integral in development of and data storage on the LNVA Mapper.

Public land access was coordinated through state wildlife management area (WMA) and federal national wildlife refuge (NWR) and preserve managers. All sampling was conducted according to special use permit requirements. We thank A. Bennett for coordination at Big Thicket National Preserve, D. Bossert and L. Gonzales at Trinity NWR, K. Brunson for coordination at Gus Engeling WMA, K. Hand for coordination at Big Lake Bottom WMA, J. Sanchez and C. Jones at the Texas Mid-Coast NWR Complex (specifically San Bernard NWR), and V. Neace for coordination at Caddo Lake WMA. Access to private lands was granted by multiple entities including the Brazoria County Parks and Recreation Department (esp. J. Jiminez), City of Sugarland (esp. J. Cordova), Ethician Foundation (esp. G. Russell), the Harris County Precinct 3 (esp. R. Belzung, J. Geisler, and J. Naivar at Kickerillo-Mischer Preserve and J. Carey at Jesse Jones Nature Preserve), and Sabine River Authority (esp. D. Williams). Numerous private citizens provided access to their properties including R. Bailey, K. Franks, and S. Ross.

This work would not have been possible without the help of our field and lab assistants who handled months of travel, hot summer days, early mornings, hordes of insects, and hours of sample processing. Thank you to Aurora Alvarez, Floyd Boyett, Sarah Bullard, Jacqueline Carlson, Danielle Davidson, Danielle DeChellis, Grey Dennis, Gretchen Frederick, John Hamilton, Taylor Hackemack, Nick Hughes, Isabel Johnson, Jazmynn Kennebeck-Miller, Story Lesher, Isabel Marzullo, Taylor McKenzie, Jason Nagro, Victoria Ontiveros, Stephen Ross, Sarah Schrock, Taylor Thomisee, Cody Turner, Mathew VanBemmel, Haley Welshoff, Roxy Wrast, and Emily Yargeau.

Internal support for the project was provided by numerous departments including the Office of Sponsored Programs (esp. N. Devino, T. Rahman, L. White, R. Warner, and E. Hayes), Travel (esp. R. Sills), Accounts Payable (esp. R. Pineda), Procurement (esp. C. Chapman and D. Bonilla), Office of Information Technology, and University Facilities Management. Debbie Bush (EIH's Web Administrator) was critical to spreading information about the project over social media. Our Business Administrator, Ms. Patti Koch, worked tirelessly through restrictions, force majeure, and a global pandemic to ensure that supplies were procured, personnel were paid, and coordinated many tasks with internal and external departments.

Finally, we would especially like to thank the Texas Comptroller of Public Accounts for their funding, support, and communication. Our project and contracts managers and our former subject matter expert were integral in the development and implementation of this project.

All sampling was conducted under Texas Parks and Wildlife Scientific Collection Permit SPR-0321-0236 and UHCL Institutional Animal Care and Use Committee Protocols 0320.001.R1-R2. Funding was provided under TCPA contract number 21-7219BG.

List of Abbreviations

AIC	Akaike Information Criterion
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
AST	Alligator Snapping Turtle (*Macrochelys temminckii*)
CPUE	Catch per unit effort (number of turtles per trap night)
BCI	Body condition index (mass as a function of midline straight carapace length)
CSE	College of Science and Engineering
DAPTF	Declining Amphibian Task Force
DBH	Diameter breast-height
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DSLSR	Days since last significant rainfall
EIH	Environmental Institute of Houston
ESA	Endangered Species Act
FAV	Floating aquatic vegetation
GLM	Generalized linear model
GPS	Global Positioning System
HW	Head width (mm)
IUCN	International Union for Conservation of Nature
LEK	Local ecological knowledge
LNVA	Lower Neches Valley Authority
max-CW	Maximum carapace width (mm)
max-SCL	Maximum straight carapace length (mm)
mid-SCL	Midline straight carapace length (mm)
mid-SD	Midline shell depth (mm)
NMDS	Non-metric multidimensional scaling
Non-LEK	Non-local ecological knowledge
outer-PW	Outer plastron width (mm)
PC	Principal component
PCA	Principal components analysis
PCR	Polymerase chain reaction
PIT	Passive integrated transponder
PL	Plastron length (mm)
pre-C	Pre-cloacal tail length (mm)
R+C+S	Red, Cypress, and Sulphur river basin metapopulation
RAD	Restriction site associated DNA
Sa+N	Sabine and Neches river basin metapopulation
SAV	Submerged aquatic vegetation
SE	Standard error
SFA	Stephen F. Austin State University
SNP	Single nucleotide polymorphism
SSA	Species status assessment
SWQM	Surface Water Quality Manual
TCEQ	Texas Commission on Environmental Quality
TPWD	Texas Parks and Wildlife Department

TSA	Turtle Survival Alliance
TT	Texas Turtles
TTWG	Turtle and Tortoise Working Group
TWDB	Texas Water Development Board
TXNDD	Texas Natural Diversity Dataset
UHCL	University of Houston–Clear Lake
USEPA	United States Environmental Protection Agency
USFS	United States Forest Service (U.S. Department of Agriculture)
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
VCF	Variant call file

EXECUTIVE SUMMARY

Long-term population studies provide critical insights into how wildlife interact and function within their ecosystems; however, studies on long-lived species, such as the Alligator Snapping Turtle (AST; *Macrochelys temminckii*), are rare as many species outlive the life cycle of a typical grant, graduate student study, and sometimes the career of the primary researcher(s). Effects of anthropogenic threats to the species, in combination with their generally cryptic behavior, have led to a perceived decline in abundance and distribution throughout the species range. Currently, the USFWS is evaluating AST for protections under the Endangered Species Act. The current study aims to inform baseline population viability of *M. temminckii* in Texas and to establish reference sites for future long-term monitoring projects. Our primary objectives are to: (1) characterize abundance and demographics, (2) assess genetic structure, (3) provide a basis for future long-term efforts, and (4) contribute to a database to serve as a baseline for future efforts.

In order to guide the site selection process and assess areas outside of those previously surveyed, we amassed a compilation of spatial and temporal data (e.g., historic accounts). We selected survey locations based on prevalence of historic accounts in a given area and observation type(s) in basins where AST occupancy is established and in basins where AST occupancy was unknown, we selected sites based on aerial habitat imagery and recommendations from local experts. During each sampling event, general site data, water quality variables, and habitat data were documented with methods similar to previous assessments. When AST were captured, we measured, weighed, marked, sexed and photographed the individual prior to release. Additionally, tissue samples were collected from each individual for population genetic analyses. We documented presence of external traits and abnormalities, developed a non-invasive protocol for metal detection, and evaluated female AST for reproductive structures (e.g., follicles or eggs). Finally, we compared our results to previous surveys and compiled additional unpublished morphometric data from key collaborators and contributors.

We conducted surveys at 34 locations representing 25 counties between April 2021 and November 2022. Overall, we were successful in detecting AST occupancy at 24 locations. Average survey effort was 18.8 ± 0.33 trap-nights and the average length of the survey reach was 1,341.9 ± 7.99 river-kilometers. Overall, we conducted sampling efforts in 10 of the 11 east Texas river basins. We were unable to conduct surveys in the San Jacinto-Trinity basin due to lack of available habitat and access permissions. Riverine habitat comprised 88.2% sites while lacustrine habitat comprised 11.8% of sites. Throughout the study, 78 AST were captured with 69 unique individuals documented over 1,558 trapping nights. Average catch per unit effort (CPUE) for all sampling events was 0.053 ± 0.0116 while CPUE for events when ASTs were captured was 0.137 ± 0.0234. Overall, CPUE, number of AST, and effort did not differ significantly between the current and previous surveys in Texas.

We demonstrate that use of local ecological knowledge can guide surveys focused on detection of cryptic or difficult to find species (such as AST) in areas where detection or occupancy has not been previously established. Overall, catch per unit effort for AST populations in Texas have not drastically changed in the past 10+ years, especially between surveys conducted > 10 and < 3 years ago. Additionally, AST in Texas appear to be most active during the Spring (February–May) and Summer (June–September) seasons, though this may be due to anecdotal observations being correlated with increased recreational activity during those times.

Based on genetic analyses, Texas AST are divided into three metapopulations within the Red, Cypress and Sulphur river basins (metapopulation #1), Sabine and Neches river basins

(metapopulation #2), and San Jacinto and Trinity river basins (metapopulation #3). Though our results show that effective population size and genetic diversity are low, it is hard to make direct comparisons to other AST populations because this is the first study to evaluate effective population size for the species. Therefore, effect(s) of anthropogenic structures, such as dams, could not be assessed. Though the extensive dam and reservoir system in east Texas may affect AST populations, it may take > 400 years before direct impacts can be assessed and future studies are needed to address this.

Morphometric data were consistent with known trends and previous studies. Specifically, Body Condition Index (e.g., midline straight carapace length and mass) was highly correlated amongst AST in Texas, though differed between sexes and proposed age-size classes. Establishment of an age-size class matrix for the species is imperative for future conservation efforts focused on specific life history stages. Here, we propose an age-size class structure that we believe accurately represents AST in Texas, though it should be further evaluated and refined to reflect the greater AST population nationwide. Additionally, further evaluation of the effects of external injuries or abnormalities, specifically epiphytic growth or external parasites, are recommended to assess overall impacts to survivorship. To our knowledge, we have compiled the first documentation of reproductive development in wild-captured female AST in Texas. Observation of presence (or absence) of specific reproductive structures suggests that AST in Texas are nesting during the Spring (April–June) season, though females may not be clutching every year.

Likelihood of AST detection was increased in areas where dissolved oxygen concentrations were near 10 mg/L, thalweg depth was > 2-meters, water temperatures were lower overall (mean = 23.31°C), in-water cover lacked large woody debris or structure, areas where substrate was primarily composed of fine materials, and in waterbodies with increased bank slope. Riverine habitats (especially those surrounded by forested riparian structure) had the highest proportion of AST detections, though further evaluation of microhabitat selection in lacustrine environments are needed. Data for active, passive, or derelict fishing gear observations and access points were not significantly correlated to AST presence, similarly to recent studies in Texas. Further evaluation of the influence of anthropogenic disturbances or use of riverine and lacustrine habitats are needed. Implementation of a newly developed protocol for detection of foreign metallic objects using a handheld metal detector was successful in locating internal metallic objects, but unable to determine the type of object. This protocol confirmed that fishing hooks could be detected using the handheld metal detector, but further evaluation of the protocols wider application and ability to identify specific metallic structures is needed.

We recommend future assessments of AST population in Texas utilize our proposed list of primary and secondary candidate long-term monitoring locations or apply our site selection matrix for selection of new locations outside of those previously established. Use of these guides will aid future efforts to evaluate questions related to the greater AST population (and metapopulations) in Texas. For resource managers to make the best recommendations for conservation measures moving forward, we recommend they consider implementation of an age-size class matrix for life history stage-specific conservation actions. Ultimately, while we were able to compile, compare, and add to the existing base of knowledge for AST in Texas, a full population viability assessment requires multiple years, even decades in the case of a long-lived species like the AST, in order to elucidate meaningful relationships. Future efforts to continue long-term monitoring surveys of AST populations in Texas will be critical in the overall conservation, protection, and, possibly, increased population sizes.

INTRODUCTION AND BACKGROUND

Long-term population studies provide critical insights into how wildlife populations interact and function within their ecosystems (Lindenmayer and Likens 2009, Clutton-Brock and Sheldon 2010). Because many of the most important ecological functions provided by a species can take years or decades to manifest, long-term studies are critical for generating baseline data to compare trends over time, ultimately allowing for improved management of a species and the ecosystems they inhabit (Lindenmayer and Likens 2009, Clutton-Brock and Sheldon 2010). However, long-term studies on long-lived species are rare as they are difficult to maintain, with many species outliving the life cycle of a typical grant, graduate student study, and sometimes the career of the primary researcher(s) (Tinkle 1979, Franklin 1989, Congdon et al. 1994, Seigel and Dodd 2000). This is particularly true of chelonians, with some species having lifespans of 100+ years (Gibbons et al. 2000). As a result, there are few long-term studies on turtles, even though they are generally long-lived, important to ecosystem services, and occur at relatively high biomass (Iverson 1982, Congdon et al. 1994, Lovich et al. 2018, Munscher et al. 2020a).

Large turtle species, like the Alligator Snapping Turtle (AST; *Macrochelys temminckii*), exhibit low recruitment, slow growth, and long generation times; life history traits which make localized populations vulnerable to exploitation (Gibbons 1987, Iverson 1991, Ernst and Lovich 2009). Within the United States, ASTs are affected by habitat loss, unsustainable harvest rates, environmental pollution, and poaching (Pritchard 1989; Sloan and Lovich 1995; Gibbons et al. 2000; Cebellos and Fitzgerald 2004; Boundy and Kennedy 2006; Ernst and Lovich 2009; Dixon 2013; Hibbitts and Hibbitts 2016; TTWG 2017, 2021; USFWS 2021). This species has historically been fished across the U.S. and, due to the increased demand of turtle meat in the overseas market, many populations are feared to have been locally extirpated (Huntzinger et al. 2019, Munscher et al. 2020a, Riedle et al. 2005, USFWS 2021).

Alligator Snapping Turtles are found in Gulf of Mexico drainages extending from Florida to Texas (Pritchard 1989, Ernst and Lovich 2009, Dixon 2013, Hibbitts and Hibbitts 2016, USFWS 2021). In Texas, ASTs are generally described as ranging as far west as the Trinity River basin (Dixon 2013, Hibbitts and Hibbitts 2016), although a fossil specimen from the Brazos River drainage suggests that it once occurred farther westward (Hay 1911). Historic and recent surveys have confirmed occupancy of the species in 57 counties of Texas (Figure 1). Alligator Snapping Turtles reside primarily in deep, slow moving fresh-waterbodies associated with rivers including oxbows, bayous, and connected lakes and ponds (Ernst and Lovich 2009, Hibbitts and Hibbitts 2016). Additionally, ASTs rarely bask, are generally nocturnal, and typically spend most of the time submerged, unlike other aquatic turtle species (Pritchard 1989, Hibbitts and Hibbitts 2016). Effects of anthropogenic threats to the species, in combination with their generally cryptic behavior, have led to uncertainty about the species population status, abundance, and distribution throughout the species range (USFWS 2021).

The distribution of a species is a central tenant of ecology and biodiversity conservation (Pagel et al. 2014). Understanding where a species should occur and where a species does occur is a natural first step in designing conservation strategies. Complimenting species distribution information, determination of population abundance and demographic trends are important components of assessing how to assign conservation status to species according to the International Union for Conservation of Nature (IUCN) criteria (Pagel et al. 2014, IUCN 2017). Prior to development and implementation of population abundance or viability analyses, it is imperative to collect and compile baseline demographic and distribution data so that changes to a species status can be tracked over long periods of time. Due to gaps in our knowledge of AST demography and outdated knowledge of distribution, baseline data collection and long-term monitoring of reproductively viable populations is needed to fully understand how to conserve this species. As a long-lived species, compilation of decades-long data is necessary in order to properly determine overall population viability in the long-term.

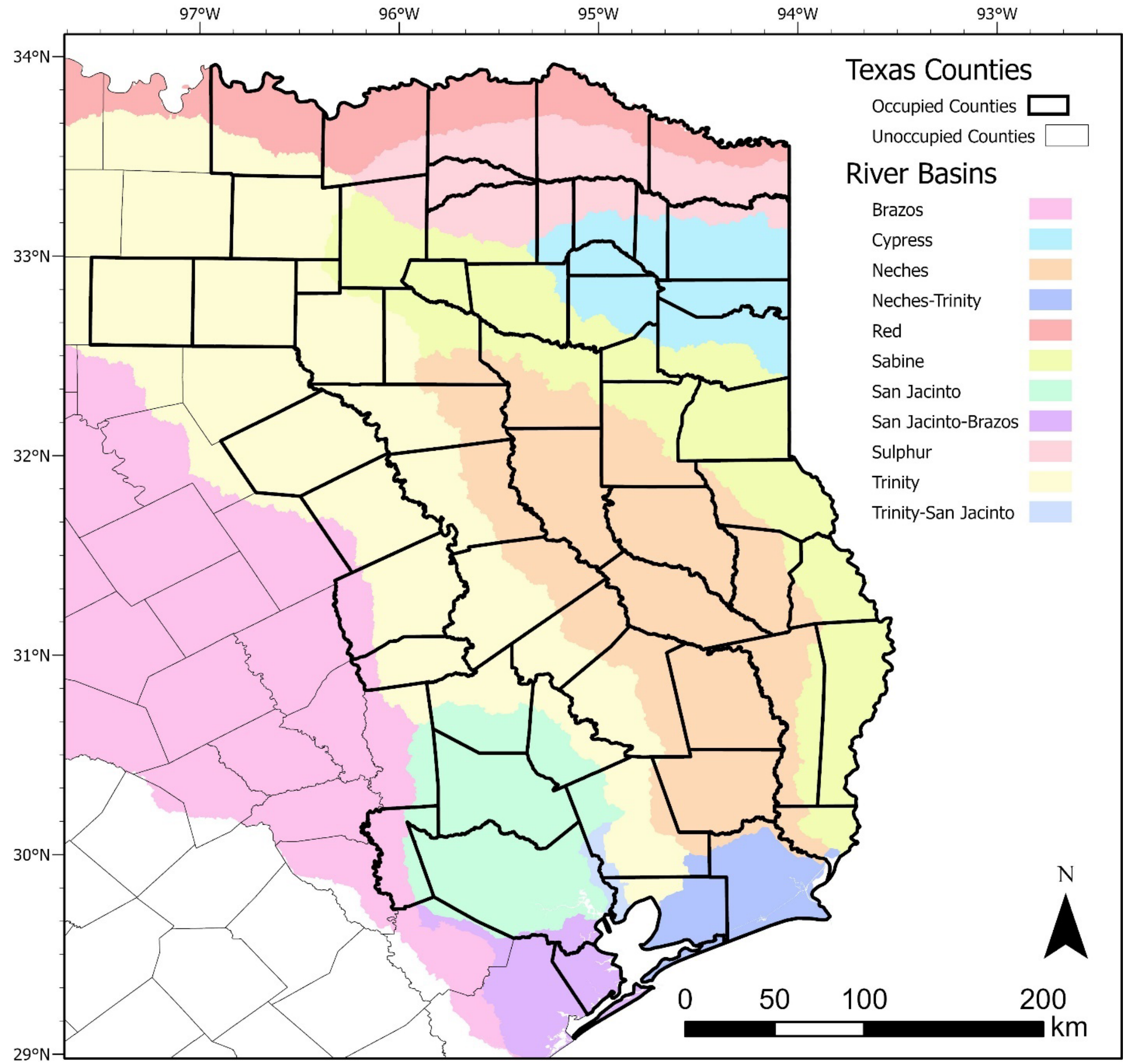


Figure 1 Current and historic Texas counties occupied (thick border) by Alligator Snapping Turtles (AST; *Macrochelys temminckii*) superimposed over east Texas river basins (TWDB 2021). Designation of occupied counties from Dixon (2013), Hibbitts and Hibbitts (2016), Baxter-Bray et al. (2021), Franklin et al. (2021), Norrid et al. (2021), USFWS (2021), and Rosenbaum et al. (2022).

Much of what is known about the species' range, distribution, and demographics in Texas is based on occurrence records and grey literature, though many organizations are working to expand our knowledge of the species. Recently, what was previously the most expansive population assessment in the state (Rudolph et al. 2002) has been updated, including recaptures of individual ASTs documented 20+ years ago (Rosenbaum et al., 2022). Additionally, localized long-term monitoring efforts are being established by groups such as Texas Turtles and the Turtle Survival Alliance. A special issue of The Southeastern Naturalist focused on peer-reviewed studies of *Macrochelys* throughout its range has been in development since 2021. This special issue will contain valuable information related to Texas AST populations from multiple groups, though a final publication date is yet to be determined. Some of the topical papers focused on Texas ASTs to be published in this special issue include updates to existing demographic data for an urban population of ASTs found in Buffalo Bayou, Harris County (Munscher et al. 2023), results of the distribution and demographic study re-evaluating the outdated assessment original to Texas ASTs (Rosenbaum et al. 2023), and examination of ASTs as a model species for evaluating the use of Local Ecological Knowledge (LEK) to guide site selection and document observations of ASTs (Gordon et al. 2023). The latter uses data compiled from year 1 of this study (described below) and builds the framework for how site selection continued in year 2.

With advances in technology, a growing base of local experts (private landowners, citizens, recreational enthusiasts, etc.) are becoming more accessible to researchers and natural resource managers. Incorporation of peer-reviewed data with grey literature and anecdotal reports from individuals with local expertise can assist with expanding our understanding of a species distribution, abundance, and, in some cases, demographics (Anadón et al. 2009, Cano and Tellería 2013, Cross et al. 2021, Madsen et al. 2020). This LEK can provide insight to potentially long-term trends in presence, absence, movements, and/or population fluctuations across a wide range of vertebrate species (Farhadinia et al. 2018, Riggio and Caro 2017, Turvey et al. 2014).

A range of definitions for LEK have been suggested (Davis and Ruddle 2010, Pauly 1995). We define "local ecological knowledge" by using the three key attributes outlined by Davis and Ruddle (2010): 1) a shared knowledge about the environment and ecosystem relationships, 2) this knowledge is developed through direct experience, and 3) the knowledge is transmitted between or among generations. In Texas, over 95% of lands are privately owned or managed with many properties having been managed over multiple generations (Lopez et al. 2014). Therefore, researchers and resource managers conducting assessments in Texas have access to a unique group of individuals with an extensive history of LEK across a wide range of habitats and species. This LEK may be particularly useful in studies focusing on long-lived and/or cryptic species, especially those under review for inclusion as part of the Endangered Species Act (ESA; Crocetta et al. 2017, Cross et al. 2021, Madsen et al. 2020).

Conservation Need

Alligator Snapping Turtles have been listed as a Threatened species in Texas since 1987 (Register 1987). In 2012, a petition to protect ASTs under the Endangered Species Act (ESA) was submitted to the U.S. Fish and Wildlife Service (USFWS) with a significant 90-day review requiring additional status information throughout the species' range (Giese et al. 2012, USFWS 2015). In 2021, the USFWS released a Species Status Assessment (SSA) with a suggestion to list ASTs as threatened under the ESA with a 4(d) ruling (USFWS 2021). Specifically, the following priority topics for ASTs have been identified throughout their range (J. Culbertson, U.S. Fish and Wildlife Service, Region 2 Species Coordinator, *personal communication*):

1. Species' biology, range, and population trends
2. Threats to the species
3. Spatial distribution and extent of threats

4. Spatial variation in demographic rates related to reproduction, recruitment, and survival
5. Personal or commercial trade
6. Habitat loss or degradation impacts to the species
7. Design of a turtle exclusion device, modified trot line techniques, etc. to reduce bycatch
8. Information to address uncertainties from the future condition analyses
9. Regulations that are necessary and advisable
10. Whether the measures outlined in the proposed 4(d) rule are necessary and advisable
11. Reasons why [they] should or should not designate habitat as "critical habitat"
12. Whether the designation of critical habitat is not prudent
13. Specific information on possible risks or benefits of designating critical habitat

Following the release of the SSA in 2021, an influx of data related to ASTs and concerns from citizens, scientists, and resource managers caused the USFWS to reconsider their original SSA, particularly in relation to their model designs and evaluation of the Mississippi West and Western sub-units (David Castellanos, U.S. Fish and Wildlife Service, Species Lead, *personal communication*). As of this report, the USFWS are still in the process of re-evaluating their original models, and data resulting from the current study may be used to help guide the modelling process (Erica Christensen, U.S. Fish and Wildlife Service, modelling specialist, *personal communication*).

Objectives

The current study focuses on major river basins (as defined by the Texas Water Development Board, TWDB) within east Texas including the Red, Sulphur, Cypress, Sabine, Neches, Trinity, San Jacinto, and Brazos rivers and their associated tributaries. Our overall goal is to locate viable populations within and outside of previously documented locations and establish reference sites for future long-term monitoring projects. This research aims to inform baseline population viability of *M. temminckii* using the following four primary objectives:

1) Characterize abundance and demographic parameters
2) Assess population genetic structures
3) Coordinate with stakeholders to provide a basis for future long term-monitoring efforts
4) Create or contribute to a database or web-based viewer that can be combined with historical data to serve as a baseline for future monitoring efforts.

Through coordination with local, regional, state, and federal partners, we aim to not only fill knowledge gaps on the natural history of this species within Texas, but provide critical baseline data within the western extent of the species range, overall.

METHODS

Site Selection

In order to guide the site selection process, we amassed spatial and temporal data, including information from peer-reviewed publications, agency reports, grey literature (reports, theses, dissertations, etc.), online community reports, and LEK. Collectively, these data are referred to as "historic accounts". We selected field survey areas based on prevalence of historic accounts in a given area, observation type(s), and included areas where AST reports were lacking but habitat suggested the possibility of presence. The following outlines how we compiled data and selected sites for trapping surveys, as well as general field surveys methods and data analyses.

Published reports and verified records (non-LEK)

We conducted a literature review specific to ASTs in Texas using Google Scholar (http://scholar.google.com) and included journal articles, reports (clinical, agency, etc.), books, natural history notes, geographic distribution notes, theses, and dissertations. Our database included information such as: source, observation location(s), observation date(s), number of ASTs reported, etc. We used distribution and range maps from books or field guides to confirm overall range and to set boundaries to identify regions where LEK should be solicited (see next section for description of LEK sources) (Dixon 2013, Hibbitts and Hibbitts 2016).

We compiled additional non-LEK records from VertNet (2021) and the Texas Natural Diversity Database (TXNDD; 2021). We extracted VertNet data using the following search terms: "Genus=*Macrochelys*" and "StateProvince=Texas". We did not include misspellings of either keyword. Specific location data for specimen reported by the University of Texas-Arlington Amphibian and Reptile Natural Diversity collection were provided as a supplement to the data extraction due to this information being excluded prior to submission to VertNet (Carl Franklin, unpublished data). Observations from the TXNDD are amassed from a variety of sources primarily as part of ongoing Texas Parks and Wildlife Department (TPWD) funded research or permitting requirements. We synthesized observations into representative temporal data points as part of a GIS shapefile and supplemental data related to each report is provided as a .PDF document. To extract data, we submitted an email to TXNDD staff requesting a compilation of all *M. temminckii* records in Texas, "regardless of county or specific location". We then reviewed the PDF and GIS shapefiles for accuracy and detail prior to entry into the historic account database.

Accounts compiled as local ecological knowledge (LEK)

We compiled observational and anecdotal accounts of ASTs from a variety of sources including: iNaturalist (https://iNaturalist.org) (2021), an ArcGIS Online Mapper developed by the Lower Neches Valley Authority ("LNVA Mapper"), social media (Facebook), and personal communications with individuals who had familiarity with ASTs.

iNaturalist is accessible to anyone capable of generating an online account and allows individuals to post photographs to a public website where proper identification can be crowdsourced and confirmed. We extracted data using the following search criteria: 1) "Species=*Macrochelys*", 2) "Location=Texas", and 3) the "Research Grade" filter activated. In order for an observation to qualify as a "Research Grade" report, it must: 1) have a date, 2) be georeferenced, 3) have photos (or sounds, if applicable), 4) not be a captive or cultivated organism, and 5) greater than two-thirds of identifiers agree on species-level ID. iNaturalist moderators are able to obscure geolocational data for species such as ASTs, where publicly accessible data may be used outside the context of research or education (e.g., poaching, black-market trade, etc.). Spatial data for all AST observations extracted from iNaturalist were obscured, so we created a personal account and used it to contact users for more accurate spatial information. We informed users that data were being used for research, and we asked them to provide specific spatial data and provide consent to use the data as part of the study. We excluded spatial data originating from reports where the user did not respond to inquiries from analyses. When multiple users generated reports of the same animal or a single user generated multiple reports for each animal observed at the same location, we consolidated this information into a single data point.

As part of a collaborative effort between private stakeholders, landowners, researchers, conservation resource managers, and local communities, an online database of AST observations was developed by the Lower Neches Valley Authority via ArcGIS Online ("LNVA Mapper"; ESRI 2021). The LNVA Mapper compiles photo-verified sighting reports of ASTs to a single repository, which is accessible by researchers and state and federal partners. For reports to be included in this collaborative effort, they were required to include: 1) a photograph, 2) GPS coordinates, 3) date and time, and 4) any additional information related to the observation (including condition, size, gender, behavior, etc.). Representatives from select entities were granted the ability to add reports as they were obtained, generating a database that updated as observations were made in real-time. To solicit reports from local experts, various agencies conducted opportunistic surveys during day-to-day operations (e.g., interactions with recreational fishermen, observations made by colleagues, etc.). Additionally, some agencies posted signage at public boat ramps in areas where AST populations were known or likely to exist and provided a reporting hotline number or email address. We used spatial and temporal data from all observations extracted from this database.

We sourced additional photo-verified reports of AST observations from social media posts to various Facebook (2021) special interest groups including "NECHES RIVER LIFE", "Texas Turtles", "Snapping Turtle Fanatics", "What kind of snake is this? North Texas Educational Group", and "Native Texas Wildlife" (accessed from 08 January 2021 through 01 October 2021). These groups include a wide range of wildlife enthusiasts, recreational fishing guides, and herpetological outreach groups across Texas. When a report was brought to the attention of the senior author, we privately contacted reporters via Facebook Messenger using a personal Facebook account. As with iNaturalist users, we informed individuals that data were being used for research and asked them to provide specific details about spatial data and to provide consent to use the data as part of the study. We generated a permanent link for each account and downloaded photographs for posterity. While many individuals were able to provide specific dates and/or locations of observations, if specific data could not be provided with certainty, we excluded them from spatial or temporal analyses.

Finally, we documented conversations with landowners, stakeholders, recreational enthusiasts, agency professionals, and other individuals with extensive LEK in each watershed, county, region, or property from January–July 2021. During communications (via phone, email, Zoom, Microsoft Teams, or in person), we asked individuals to provide spatial and temporal data related to observations of ASTs in east Texas. If communicators had access to photographs of observed specimen(s), they were provided, otherwise all communications were based on recollections or personal experience. In some cases, communicators were not able to provide specific dates or seasons for observations but could provide specific coordinates. In other cases, communicators were able to provide exact dates and details of the observation, but only general areas or non-specific locations. To that end, we used historic accounts provided by personal communications in only spatial or temporal analyses, but rarely both.

Site selection matrix

To aid resource managers in selection of future candidate AST monitoring locations outside of those previously studied, we developed a site selection matrix applying common considerations used for identification of candidate site locations and tested its applicability using results from trapping surveys in this study. Considerations were split into seven categories including: reliability of the observation(s) related to a prospective sample location, quality of the spatial data associated with the proposed location, age of the observation or sighting in relation to the time at which the location was being considered for use, physical accessibility to the proposed location, general site characteristics within 250 m upstream and downstream of the proposed location, potential for tampering with gear or equipment, and whether or not site access permission could be attained (see Appendix A for full selection matrix). Each category was assigned a range of scores based on our perceived importance to the potential for the proposed location(s) to result in detection of ASTs. For all categories, the highest score was considered the most impactful or important. Reliability of the observation or sighting was perceived as the most important consideration and assigned a range between zero and five. Quality of the spatial data, age of the observation, physical accessibility, and general site characteristics were considered equally important and, thus, assigned a range between zero and three. Tampering potential and access permission were considered least important of the seven categories (though still necessary considerations when determining candidate field locations), and were assigned a range between zero and two. With this range of scores, location(s) that would be considered to have the highest likelihood of AST detection should have a cumulative score of 21 while location(s) considered to have the lowest likelihood of AST detection would have a cumulative score of zero or near zero.

Prior to final field site selection, a subset of the historic account locations with the highest likelihood of AST detection were scored and the resulting scores were used in consideration for addition of the site(s) to the sample design. Ultimately, all historic account locations, regardless of source and/or quality of the spatial or temporal data, were also scored using the matrix.

Final site selection

Across all data sources, we compiled spatial data at varying resolutions (e.g., GPS coordinates, property name, waterbody name, road crossing, city, county, basin, state). In instances where enough information was not provided to reliably produce a spatial datapoint for mapping, we removed the report from the historic account database prior to site selection. We selected field sites based on proximity to historic accounts, number of observations made in each area, accuracy of the observation(s), and accessibility to the waterbody (see description of Site Selection Matrix below). To expand survey locations outside of areas associated with previous or ongoing population assessment(s), we flagged spatial data related to ongoing surveys and removed them prior to site selection. In areas where verified historic accounts were lacking, we selected candidate sites using aerial imagery and topographic maps. Prioritized habitats included areas which were accessible along banks or by boat, average water depth (near or > 1-meter depth), riparian tree canopy coverage, and presence of log jams, large woody debris, or other preferred structures (e.g., sandy beaches or bars, increased channel sinuosity, presence of small tributaries or creeks within the intended survey reach, etc.). In order to determine a list of final candidate field sites, we used a combination of cumulative score for the location(s) based on criteria from the site selection matrix, communication and coordination with landowners and stakeholders in areas of known or potential AST occupancy, visual inspection of aerial imagery at and around the proposed location, and, in some cases where multiple potential locations were identified in close proximity to one another, field reconnaissance in the proposed survey area(s).

Once candidate field sites were identified, they were compared to river basin boundaries from the Texas Water Development Board (TWDB 2021) to ensure even distribution of site locations within each basin. In basins with numerous historic accounts (Neches, Sabine, San Jacinto, Trinity), we flagged historic accounts related to recent or ongoing surveys and removed them prior to site selection. In basins where AST occupancy had not been recently documented

(Cypress, Red), we coordinated with other researchers to select sites with highest potential for capturing ASTs in addition to using historic accounts. In basins where occupancy was unknown or undocumented (Brazos, San Jacinto-Brazos, Trinity-San Jacinto), we used habitat structure based on aerial imagery from Google Earth Pro (Google Inc.) and recommendations from local experts.

Assignment of priority categories for future assessments

Trapping surveys were conducted so that no site was sampled more than once in the same season of a calendar year, though they may have been sampled in the same season between calendar years. Seasons were delimited as: Spring (February-May), Summer (June-September), and Fall/Winter (October-January) (survey methods follow).

To guide level of prioritization of sites for future assessments, we assigned each site a final category of "Primary", "Secondary", "Exploratory", or "Undetermined" (Table 1). In instances where a site could only be sampled once due to extenuating circumstances (e.g., logistical issues, landowner restrictions, effects of flooding or drought, etc.), it was assigned an Undetermined status. If a site did not result in an AST capture after two trapping events in two different seasons, it was classified as an Exploratory site and was not sampled further. If a site resulted in capture of at least one AST during three consecutive trapping events, it was flagged as a Primary site and sampled up to six times throughout the duration of the study. If a site resulted in capture of at least one AST during one of two consecutive trapping events, but a third event could not be conducted due to extenuating circumstances, it was assigned a status of Secondary. Additionally, if a site resulted in capture of at least one AST during one of three consecutive trapping events, it was assigned a status of Secondary.

Table 1 Categories assigned to each site in the current survey. These categories are meant to aid in prioritization of future survey locations for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) population assessments.

Category	Number of events	Assignment criteria
Primary	Up to six	- Sites resulting in capture of at least one AST during three consecutive trapping events - Sites resulting in capture of at least one AST during two of three consecutive trapping events, but a fourth event could not be conducted due to extenuating circumstances (e.g., logistical issues, landowner restrictions, effects of flooding or drought, etc.) - Sites resulting in capture of at least one AST during two of four consecutive trapping events, but no other sites meet criteria for Primary status
Secondary	Up to three	- Sites resulting in capture of at least one AST in two of three consecutive trapping events where a fourth event could be conducted but resulted in no additional AST capture(s) - Sites resulting in capture of at least one AST in two of three consecutive trapping events where a fourth event could not be conducted due to extenuating circumstances, but a Primary site was already established within the basin - Sites resulting in capture of at least one AST in one of two consecutive trapping events, but a third event could not be conducted due to extenuating circumstances - Sites resulting in capture of at least one AST in one of three consecutive trapping events
Exploratory	Up to two	- Sites resulting in no capture of AST across two consecutive trapping events
Undetermined	One	- Sites resulting in only one trapping event due to extenuating circumstances

If a site resulted in capture of at least one AST during two of three consecutive trapping events, it was flagged as a candidate Primary site and a fourth trapping event was conducted, when possible. In instances where a fourth event could not be conducted, and no other Primary sites were previously designated within the same basin, the site was assigned a Primary status. In instances where a fourth sampling event could not be conducted, and at least one other site within the same basin was already assigned as Primary site, the site in question was assigned a status of Secondary. In instances where multiple sites within a given basin met this criterion, the site with the largest total number of AST captures was assigned a status of Primary and all other sites were assigned a status of Secondary. In some cases, sites with fewer total AST captures across three consecutive sampling events were able to be sampled a fourth time, while the site with the highest total number of AST captures could not. If the fourth event resulted in no AST captures, these sites were assigned a status of Secondary. Finally, if a site resulted in at least one AST capture in at least two of four consecutive trapping events, and no other sites met the requirements for Primary status, the site in question was assigned as a Primary site for that basin.

Field Methods

Field data collection during each sampling event included general site characteristics (including water quality and habitat data), observations of anthropogenic activities or impacts, results of trapping surveys, and individual capture data on Alligator Snapping Turtles and bycatch.

Small-scale habitat and anthropogenic stressor data collection

All environmental and water quality data were collected following protocols outlined in the Texas Commission on Environmental Quality (TCEQ) Surface Water Quality Monitoring (SWQM) Manuals (TCEQ 2012, 2014), unless otherwise noted. Habitat data were collected following methods outlined in Rudolph et al. (2002).

General site characteristics were collected at all sites, including sample date(s), macrohabitat type along the sampling reach (estuarine, emergent, riverine, ponded, lake, forest/shrub), and environmental conditions. Environmental conditions including arrival time, arrival air temperature, a visual estimation of percent cloud cover, wind speed, wind direction, departure time, and departure air temperature were recorded for each sampling day. When possible, stream flow at the time of arrival for the trap set day was recorded from a coinciding USGS Gage (Texas Water Dashboard; https://txpub.usgs.gov/txwaterdashboard/ index.html). Days since last "significant" rainfall (DSLSR) were recorded in relation to the trap set day. "Significance" levels varied by site but were generally set to > 0.10" total accumulated precipitation for the day.

Physical habitat data were recorded within a 5-m radius originating from the center of each trap. Overall habitat type (run/glide, pool, oxbow, reservoir, or other), number of woody stems > 1-inch diameter breast height (DBH) rooted in the bank, and bank profile category (flat: < 5°, gradual: 5–30°, steep: 30–75°, near vertical: > 75°, vertical: near 90°, or undercut > 90) were documented. When applicable, aerial canopy percent cover was estimated and if a tree was present as part of the canopy within the 5-m plot, distance to the tree and tree species (to the lowest taxonomic level) were recorded (Figure 2). Additionally, at the mouth of each trap, canopy cover was quantified using a spherical crown convex densiometer (Mills and Stevenson 1999) (Figure 3). In-water habitat data including habitat type, percent cover for each type, and 5 random water depths were recorded within the 5-m radius plot. In water cover types included submerged aquatic vegetation (SAV), floating aquatic vegetation (FAV), woody debris, root wads, leaf pack, substrate cobble-sized or larger, artificial cover, undercut banks, vegetation, or other. Thalweg depth (m), wetted width (m), width of the unvegetated corridor (m), and channel flow status were documented along linear transect perpendicular to stream flow. Thalweg depth was determined as the deepest part of the channel where flow passes through and was measured using a marked sounding pole or handheld depth sounder. Wetted width and width of the unvegetated corridor (m) were recorded using a rangefinder. Channel status was calculated as

$$C_{status} = \frac{W_{wetted}}{W_{corridor}} \; x \; 100$$

where C_{status} = channel status, W_{wetted} = wetted width, and $W_{corridor}$ = width of the unvegetated corridor. Flow status was assigned a category based on percentage of exposed substrate. Categories included high (< 5% of substrate exposed), moderate (5-25% of substrate exposed), or low (> 25% of substrate exposed). Substrate type at the trap location and structure immediately surrounding the trap (within 1-m) were also documented. Substrate types included detritus (leaf litter, sticks, etc.), fines (particle size < 0.06 mm), sand (0.06–2.00 mm), gravel (2.01–60 mm), cobble (6.0–25.0 cm), boulders (25.1–45 cm), or bedrock (typically unbroken, large sections). Structure types included submerged vegetation, floating vegetation, overhanging vegetation, logs, roots, undercut bank, or other and presence was noted as none, sparse (< 25%), common (25-75%), or abundant (> 75%).

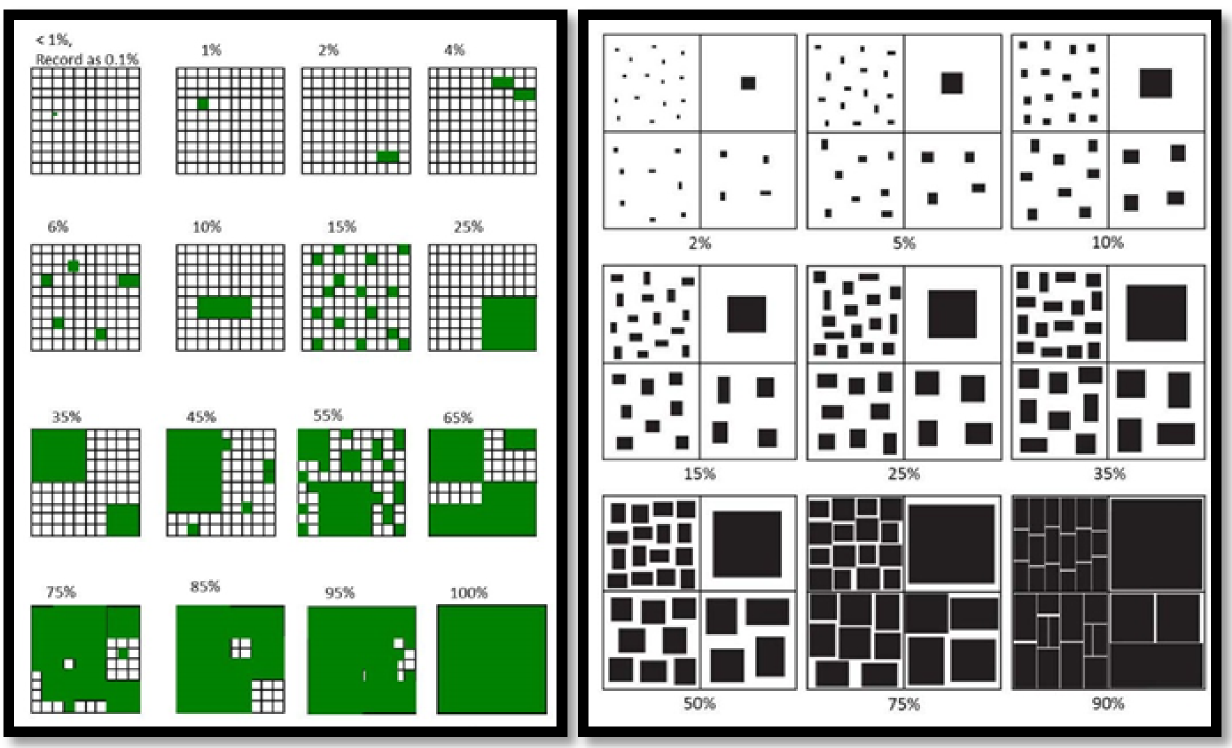


Figure 2 Examples of guides used for percent cover estimates.

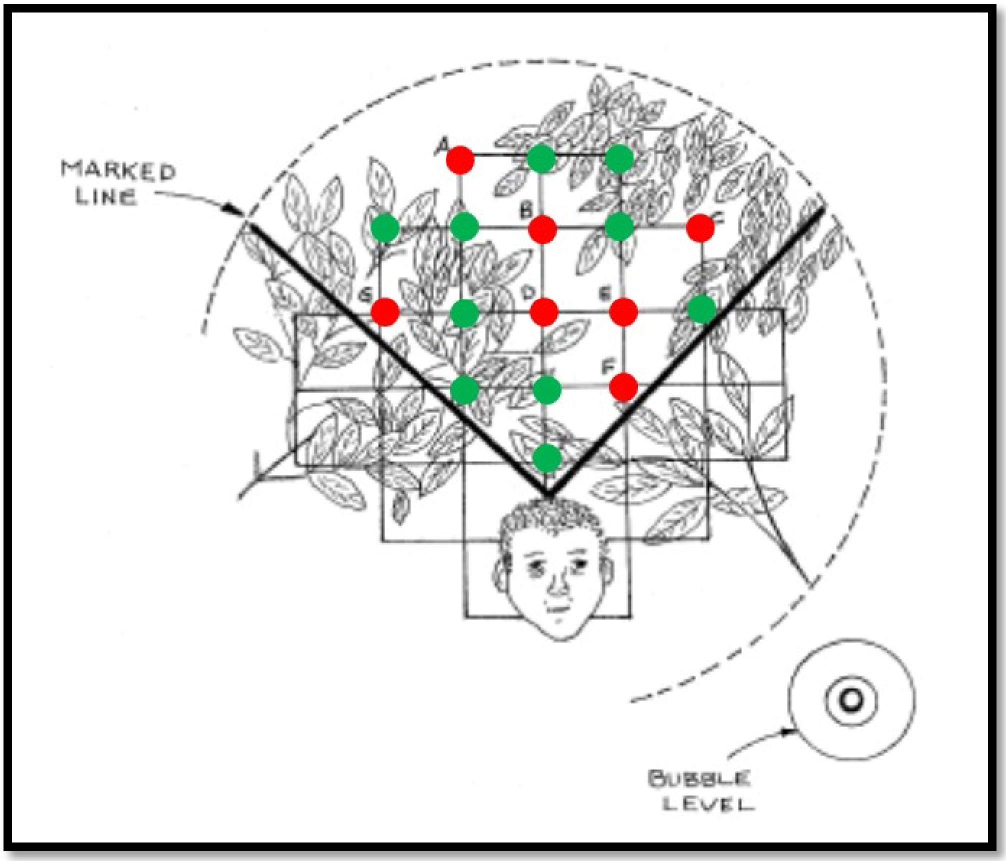


Figure 3 Example of canopy cover estimate calculated using a spherical crown convex densiometer (Mills and Stevenson, 1999). Green dots represent intersections of gridlines with canopy vegetation ($n_v = 10$). Red dots represent gridline intersections without canopy cover touching them ($n_e = 7$). Total percent cover in this example = $[n_v / (n_v + n_e)] = 10 / 17 = 58.8\%$.

Water quality variables were recorded using a multiparameter sonde (YSI ProDSS Multiparameter Digital Water Quality Meter, YSI Inc., Xylem Inc., Yellow Springs, Ohio) and measurements were recorded at 0.3 m from the bottom. Variables included: collection time, total depth (m), measurement depth (m), temperature (°C), specific conductance (µS/cm), dissolved oxygen (percent saturation and mg/L), and pH (standard units). If total depth was greater than 1.0 m, a complimentary set of water quality variables were recorded at 0.3 m from the surface. Water transparency (or “clarity”, m) was recorded using a 1.2 m Secchi tube.

Anthropogenic influences or potential stressors were visually observed and quantified along the reach including: active and passive fishing gear, recreational use, and human access points (e.g., residential and public docks and ramps). Active and passive fishing gear types included hook and line, trotlines (including juglines), limblines, nets, and traps and were documented throughout the entire study period. Trotlines and juglines could not be differentiated because we were unable to legally confirm how lines were connected underwater without disturbing them. Total counts for each active or passive fishing gear type were documented within the trapping area and we attempted to note if gear was derelict whenever possible (although passive gear was not disturbed to confirm their status). Beginning in January 2022, we began counting access points within the survey area. We used Jenks Natural Breaks Optimization analysis of fishing gear data from occupied sites to determine categorical assignments for “low”, “moderate”, and “high” anthropogenic pressure observed at each site.

Trapping survey methods

Photographs were taken at each trap location and included upstream, downstream, left bank, and right bank orientations and a photo of the trap after it was set. In instances where up and downstream could not be discerned (e.g., in reservoirs), photos were taken in orientation to the bank (left, right, water, and bank). Trap location coordinates (decimal degrees, datum WGS84) were recorded at each trap location along with trap dimensions (length and hoop diameter), mesh size (2.54–7.62 cm bar length), mouth shape (flat or round; Figure 4), distance to shore (m), and bait type.

Figure 4 Example of flat (left) versus round (right) hoop trap mouth types used in Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in east Texas. Red outlines represent overall mouth shape. White towel used as background in right image to aid in contrast.

We followed trapping methods described by Rudolph et al. (2002), Munscher et al. (2020a), and Rosenbaum et al. (2022). To summarize, we set a series of 8-10 baited hoop traps in water near or > 1 m in depth for 2–3 days (1–2 trap nights). Traps were set approximately 100 meters apart, unless habitat otherwise prohibited (Figure 5). Traps varied from 91.4–121.9 cm in diameter and 182.9–243.8 cm in length. We baited traps with frozen, cut fish of one of the following four species: *Ictalurus punctatus* (Channel Catfish), *Ictiobus bubalus* (Smallmouth Buffalo), *Oreochromis aureus* (Blue Tilapia), or *Cyprinus carpio* (Common Carp) contained in a PVC chamber. Traps were equipped with floatation devices, multi-lingual signage containing permit information and a phone number, and secured using either T-posts, rebar, anchors, ropes, or some combination based on site conditions (Figure 6). Traps were set with a portion of the trap above water (equipped with flotation devices) to allow captured organisms to breath and checked at no more than 24-hour intervals. Non-target species and other bycatch were identified to the lowest taxonomic level, measured (mm), sexed (when possible), photographed, and released at the trap location. Measurements varied by animal type. For fish and alligators, we recorded total length (tip of snout to tip of tail). We recorded straight midline carapace length for turtles and carapace width (edge to edge, including spines) for crabs. After each trapping session, total number of traps, cumulative effort (number of trap nights x number of traps set), total number of AST captures, total number of turtles captured and catch per unit effort (CPUE, number of turtles per trap night) for each were calculated.

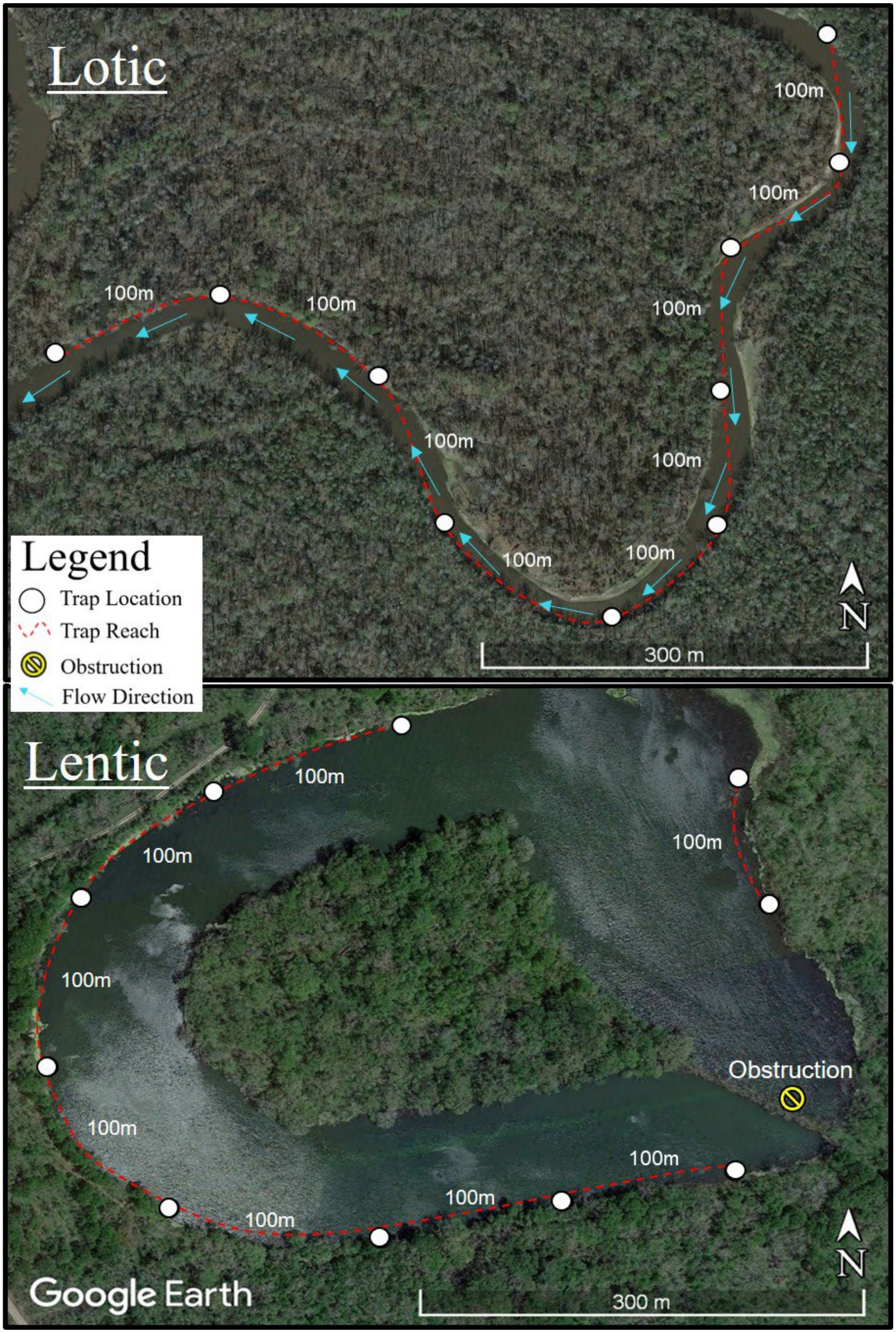


Figure 5 Example of trap distribution in lotic (top; e.g., riverine) and lentic (bottom; e.g., lacustrine) habitats sampled during Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in east Texas.

An innate conservation concern about state-wide assessments is the 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 (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 solutions included high-pressure water, a 10% bleach solution, a phosphate-free cleaning solution, or 70% ethanol.

Figure 6 Example of hoop trap set in open water. Trap secured with rebar at front and back ends, floatation device (pool noodle) visible on right-most hoop of trap.

Alligator Snapping Turtle data collection

Morphometrics

For each AST captured, we measured midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), midline shell depth (mid-SD), plastron length (PL), outer plastron width (outer-PW), and pre-cloacal tail length (pre-C) (Figure 7). All measurements were recorded in millimeters (mm). When possible, individuals were weighed (kg), marked for future identification (Figure 8), sexed, and photographed prior to release. We notched the marginal scutes of each turtle with an 8" hacksaw following a systematic pattern similar to that developed by Cagle (1939). Each individual was equipped with a passive integrated transponder (PIT) tag (APT12 PIT tag, Biomark, Boise, Idaho) injected with a sterile N125 needle and MK10 implanter into the posterior inguinal interstitial tissue (Buhlmann and Tuberville 1998). Lure color (red, pink, pinkish-grey, grey, white, clear, or mottled) of the anterior horn on the lingual lure was documented at the time of capture following Glorioso et al. (2023) (Figure 9). Any external injuries or abnormalities (shell deformity, sloughing scutes, lesions, missing limbs, presence of parasites, missing eye, or other) were noted and photographed, when possible. We also noted presence or absence of supramarginal scutes, shell algae, and estimated counts of external parasites, when present. All turtles were released within 1–2 hours of removal from the trap.

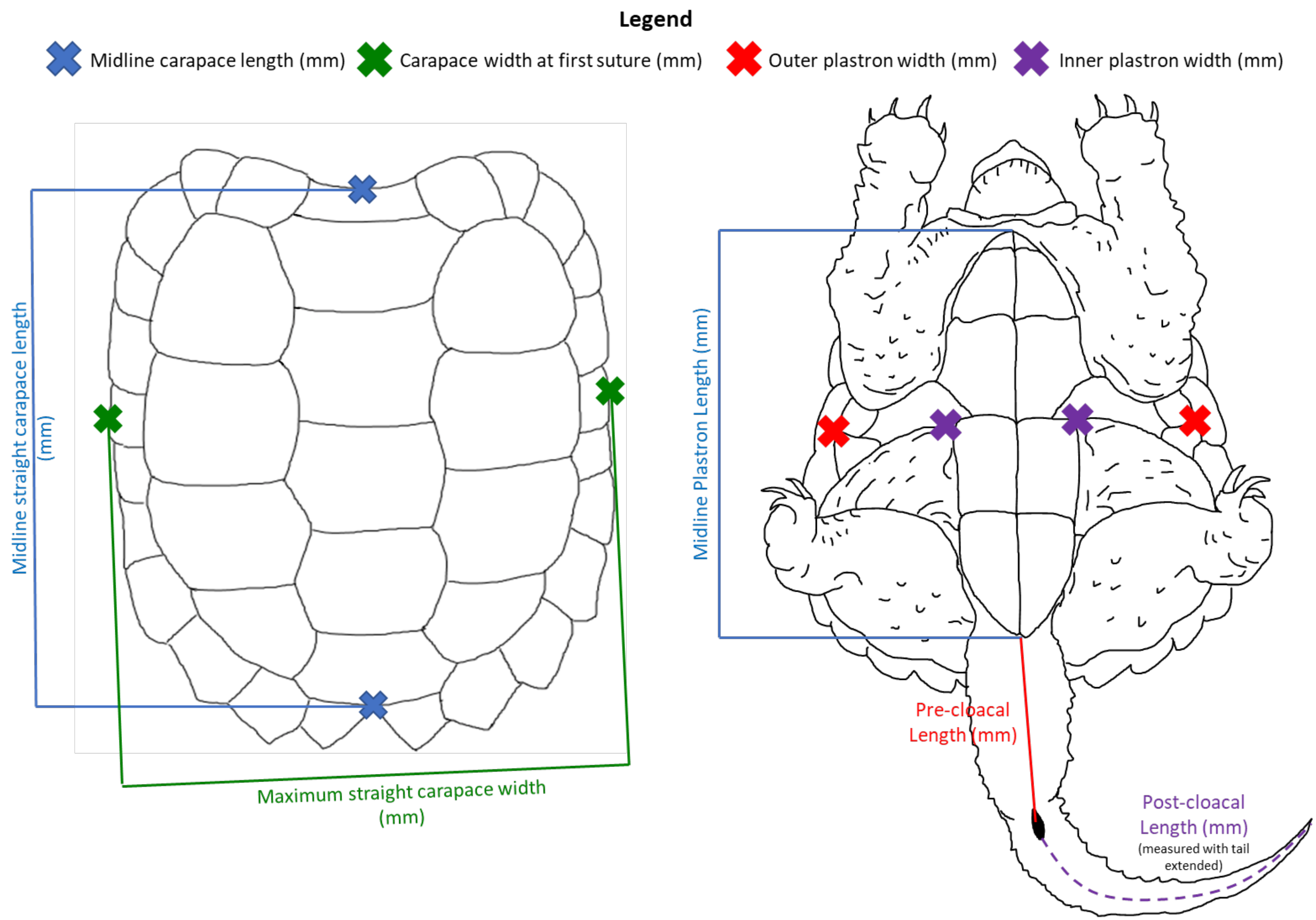


Figure 7 External carapace (left) and plastron (right) measurements recorded for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in Texas. Illustration by I. Marzullo.

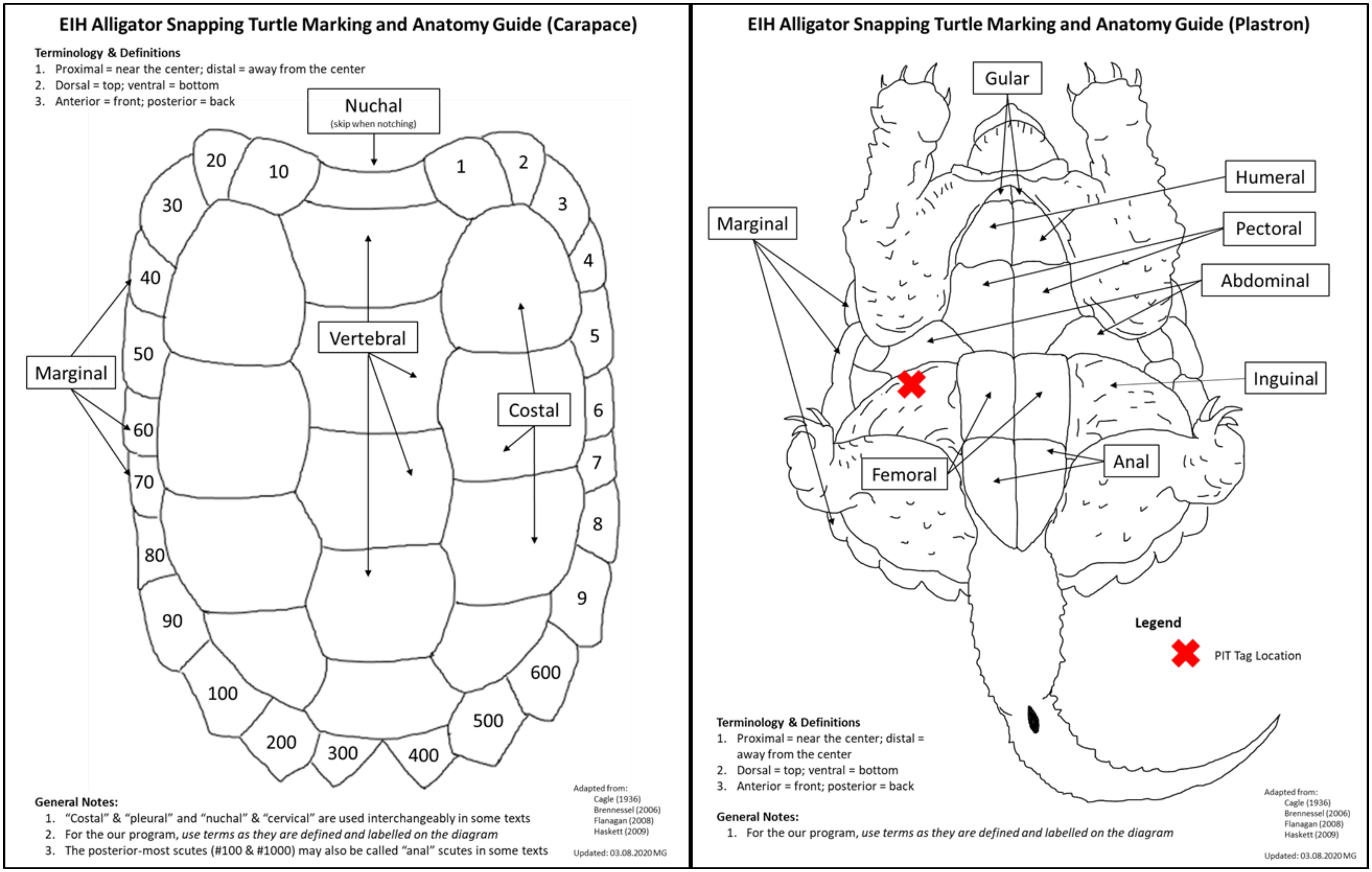


Figure 8 External notching pattern (left), location of passive integrated transponder (PIT) tag injection site (right), and anatomical terms (both images) used for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in Texas. Illustration by I. Marzullo.

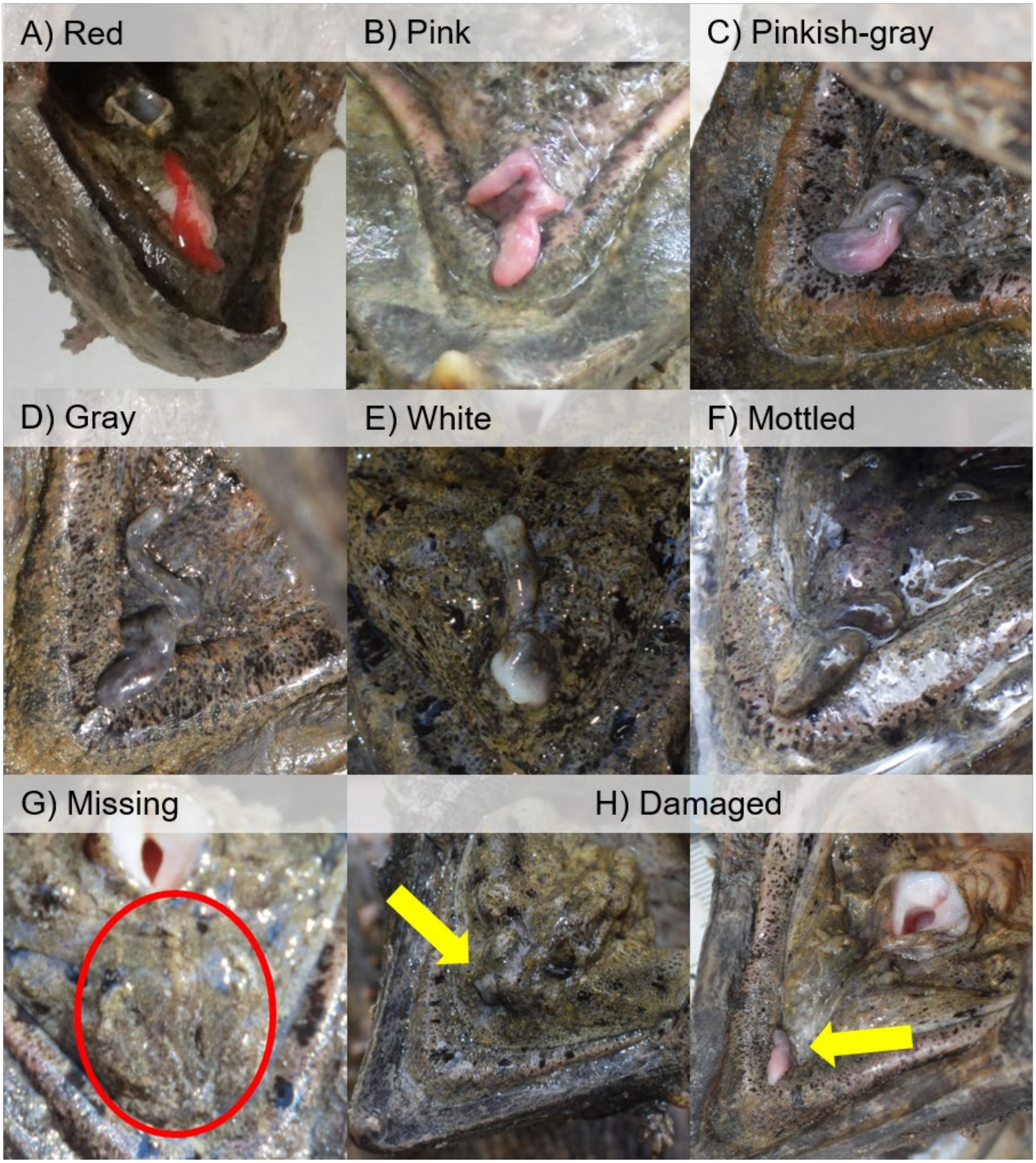


Figure 9 Standardized Alligator Snapping Turtle (AST; *Macrochelys temminckii*) lingual lure colors and conditions based on anterior horn coloration. Red circle denotes location of missing lure (panel G); yellow arrows denote damaged lures (panel H: missing anterior and posterior horns in left; posterior horn missing in right). Photo credits: B. Glorioso (USGS, Lafayette, LA, USA), M. Gordon, and L. Pearson (University of Southern Mississippi, Hattiesburg, MS, USA). From Glorioso et al. (2023); lingual lure data from turtles captured in Year 1 of the current study were included in this publication.

Tissue sample collection

Tissue samples were collected from each individual, when possible. We prioritized collection of whole blood, but when blood samples were not attainable, a skin biopsy was collected instead.

Blood samples were collected for genetic analyses from the caudal sinus or dorsal coccygeal vein using a sterile needle (21–25 gauge, 19.05–38.10 mm length) attached to a sterile 3 cc syringe (Mans 2008) (Figure 10). Target volume for each sample was 3.0 mL, but actual amount collected depended on overall body size of the individual (< 0.8% of total mass; Perpinan 2015, Adamovicz et al. 2020). Blood samples were transferred to a 4 mL sodium-heparin coated vacutainer and stored on wet ice (< 48 hour holding time) prior to freezing in the lab (-80°C). When insufficient volumes of blood were collected, a skin biopsy sample was collected from the webbing between digits 4 and 5 on the posterior left foot using a sterile 5 mm biopsy punch. Biopsy samples were transferred with sterile forceps to a cryogenic vial pre-loaded with 1 mL of 70% ethanol and stored on wet ice prior to freezing in the lab (-80°C). Samples were stored at UHCL (-80°C) and shipped to Tangled Bank Conservation (Asheville, North Carolina) for analyses during Year 2.

Figure 10 Types of tissues collected for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) population genetic analyses. Blood was collected from the caudal sinus or dorsal coccygeal vein (top) and skin biopsy samples were collected from the webbing between digits #4 and #5 on the posterior left foot (bottom). Photo credits: R. Belzung (Harris County Precinct 3).

In addition to samples collected by UHCL, researchers from Stephen F. Austin (SFA) University, the U.S. Department of Agriculture Forest Service (USFS), the Turtle Survival Alliance, and Texas Turtles also contributed tissue samples for the overall genetic assessment. Samples provided by SFA and the USFS were collected similarly to the methods described above, but whole blood was immediately transferred to a cryogenic vial with Longmire's solution as a buffer. Longmire's solution was made with 3.03g TRIS, 9.31g EDTA.Na_2, 2.5g SDS, and 250mL water. Samples provided by TSA and Texas Turtles were collected during collaborative sampling events at sites currently being routinely trapped by each group as part of long-term monitoring efforts.

Ultrasonography

To evaluate reproductive status, females (or individuals of middling size classes with unknown sex designation) were examined using ultrasonography to determine reproductive state and assigned one of the following developmental categories: quiescent (no eggs or follicles present), developing (only follicles present), pre-nesting (eggs present), and atretic (no eggs present, atretic follicles observed) similar to Rostal et al. (1990) and Moss (2010). If the animal had already been held for over one hour, or was obviously distressed during handling (e.g. having difficulty breathing, biting at personnel, etc.), ultrasound was not performed. Females were placed in dorsal recumbency on a flat surface and each inguinal cavity was examined for presence of follicles and/or eggs using a Sonosite Vet-180Plus equipped with a C11 micro-convex linear transducer (Sonosite Inc., Bothell, Washington) (Figure 11). A water-based coupling gel was applied to the inguinal tissue and the transducer was oriented in a craniomedial direction for observation of reproductive structures. Maximum follicle diameter (cm) was recorded for each side of the body cavity using internal electronic calipers built into the machine. Up to three follicles were measured on each side and photographs of follicles with and without internal caliper stamps were stored. If eggs were observed, maximum length and width of the outer shell was recorded, as well as yolk diameter. We also noted if no follicles or eggs were detected on one or both sides.

Figure 11 An ultrasound assessment of a female Alligator Snapping Turtle (AST; *Macrochelys temminckii*) during a collaborative sampling event in east Texas (left); ultrasound transducer placed in craniomedial orientation of left inguinal cavity (top right); example imagery from Vet-180Plus portable ultrasound unit (bottom right). Photo credits: C. Franklin (Texas Turtles).

Metal detection

We developed a protocol to evaluate metal detection for estimation of the frequency and occurrence of ingested foreign metallic bodies. We used a handheld metal detector (Garrett Pro-Pointer AT) due to its portability and ability to be used for both broad detection over large areas and point detection for deeper and more precise location of metallic objects. Prior to implementation in the field, we tested the capacity of the metal detector to detect metallic objects while on land and working from an aluminum watercraft. During all tests, the metal detector was able to differentiate between foreign metallic objects and the ground or hull of the vessel.

For each turtle captured, when time and conditions allowed, we performed a visual inspection over the individual's extremities, abdomen, mouth, throat, and cloaca for externally visible metallic objects. Before scanning the individual, the area underneath was scanned with the metal detector using broad detection for any interference which may result in a false positive detection(s). We first scanned the individual with it resting on its plastron and oriented carapace-side up, and the metal detector was swept along the carapace, dorsal surfaces of the limbs, tail, and head, and around the dorsal and side areas of the neck. We then put the individual in dorsal recumbency (e.g., upside down), and scanned the soft tissues along the plastron, abdomen, ventral portions of the limbs, tail, cloacal region, and neck. In general, broad detection was used for large or bony areas and extremities, while point detection was used for gently assessing soft tissue such as around the neck, cloaca, and abdomen (Figure 12a). If metal was detected, point detection was used for a more precise location, and the tissue immediately surrounding the area was re-checked to confirm negative detection. If a metallic object was detected, we used a portable ultrasound (Sonosite Vet-180Plus) equipped with a C11 micro-convex linear transducer (Sonosite Inc., Bothell, Washington) to visually confirm the presence and potential identity of the object, documenting its location, size, depth, and orientation (Figure 12b).

DNA Extraction, Library Preparation, and Sequencing

We extracted DNA for sequencing using Qiagen DNeasy Blood & Tissue kits following the standard protocols for muscle tissue, blood, and skin clip samples. We quantified the amount of DNA extracted using a Qubit 3.0 Fluorometer (Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts). We constructed 3RAD libraries for sequencing following a previously established 3RAD protocol (Bayona-Vásquez et al. 2019) to produce dual-digest RADseq libraries. The protocol includes, in order, a double enzyme digest, adapter ligation, limited cycle PCR, then a 1.2X Sepure SpeedBead cleanup (Rohland and Reich 2012). We used ClaI, BamHi, and MspI enzymes (New England Biological, Ipsqich, Massachusetts) during the digestion step. To allow for sample pooling, we barcoded each sample with internal dual indices using i5 and i7 iTru adapters (Glenn et al. 2019). We visualized the libraries on a gel, then quantified and pooled them to 100ng/µL in pools of 48 individuals. After pooling, we removed small fragments using a 1.2X SpeedBead cleanup. We size selected the pooled library 400-600bp using a Pippin Prep (Sage Science Inc., Beverly, Massachusetts). After a final quantification, we sent the pooled library to Genewiz (Azenta Life Sciences, South Plainfield, New Jersey) for sequencing. Finally, we sequenced the individuals on a Illumina NovaSeq platform with 150bp Paired End reads.

Figure 12 A) Point-detection scan using a metal detector (Garrett Pro-Pointer AT) on the ventral portion of the neck of an Alligator Snapping Turtle (AST; *Macrochelys temminckii*) to determine the precise location of a positive metallic foreign body detection. B) Scan using ultrasonography to visualize and confirm type of object originally detected with the metal detector.

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 2021). 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. In all instances, averages are presented as ± 1 standard error (SE) followed by range (minimum to maximum value) in parentheses. For all regressions, R^2 was calculated as a representation of the proportion of variance between variables. Boxplot boxes show inclusive 25th and 75th 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 trapping events). Sites were determined to be "Occupied" if one or more trapping events resulted in capture of one or more ASTs. Sites with no captures of ASTs across all trapping events were assigned a final status of "No detections". Based on the results for population genetic analyses, subsequent analyses of habitat associations, morphometrics, etc. were first tested between identified metapopulations (as determined by genetic similarity), as opposed to basin of origin, before being pooled for overall analyses.

Current and previous survey comparisons

Catch per unit effort and morphometric data

We compared number of AST reports, total number of ASTs captured, and CPUE from the current study with previous surveys by age of the survey (< 3 years, 4-9 years, and > 10 years) and season (spring, summer, fall/winter) using the data provided in Appendix B. We excluded CPUE = 0.00 and, when season was unknown, we excluded the corresponding CPUE from seasonal analyses.

Age-Size class structure

We performed a literature review and developed a proposed age-size class structure to test in the field as an attempt to aid resource managers with future conservation efforts targeted to specific age classes for ASTs. Previous assessments have designated individuals as adults, sub-adults, juveniles, or hatchlings but to our knowledge, no size class has been confidently associated with these assignments (Pritchard 1989; Rudolph et al. 2002; Fitzgerald and Nelson 2011; Riedle 2014; Munscher et al. 2020a, 2023; Rosenbaum et al. 2022). In many cases, histograms of individuals of middling sizes have shown "unknown" or "juvenile" sex assignments interspersed with "male" and "female". We compiled observances of clearly defined sexually dimorphic characteristics from the literature and their associated size classes to develop our age-size class structure matrix. In the field, technicians recorded their perceived age class (hatchling, juvenile, sub-adult, adult) and sex of the individual. We were conservative when assigning sex, especially to individuals of middling size classes, and an individual was assigned as a "U" if sex could not be obviously determined using external sexing characteristics (e.g., visual observation of cloaca extending past edge of carapace or increased ratio of pre-anal tail length to straight carapace length, especially in relation to males) or if no follicles or eggs were detected using sonography. If follicles or eggs were detected, sex was updated to F (female). For males, we did not have access to a secondary sexing technique, except in instances where the male everted his sexual organ.

Body condition index

We calculated body condition index (BCI) using the following calculation

$$BCI = \frac{\log(mass)}{\log(SCL_{mid})}$$

where log(mass) represents the base-10 log transformed value for mass (in kg) and $\log(SCL_{mid})$ represents the base-10 log transformed value for midline straight carapace length (in mm) (Jakob et al. 1996, Moore et al. 2013, Tappmeyer 2019). Body condition was compared between individuals reported in the current study and historic captures, metapopulations, and sexes. For hatchlings, max-SCL was used to calculate BCI when mid-SCL was not available.

Alligator Snapping Turtle density estimates

Density of AST for each event (D_e) at occupied sites were calculated as total number of turtles per river-kilometer (r-km) using the following formula:

$$D_e = \left(\frac{Number\ of\ AST\ captured}{Reach\ length\ in\ kilometers}\right) * 1{,}000$$

We then calculated average AST density across all sites and events and average AST density only for events with AST captures.

Population genetics data analyses

Population genetics 3RAD bioinformatics and data clean up

We first removed any individuals that did not have more than 1,000,000 raw reads returned from the sequencer. Next, we removed adapter sequences, filtered reads, clustered reads into de novo RAD loci, aligned reads to the RAD loci, called SNPs, filtered SNPs, and generated genotype files using *ipyrad* version 0.9.58 (Eaton and Overcast 2020). We used the default settings for *ipyrad* except for the minimum depth required to call a base (10X, parameter file line numbers 11 and 12) and clustering threshold (0.88, parameter file line number 14). We output a variant call file (VCF) from *ipyrad*, which we used for subsequent analyses. To minimize the effect of missing data on analyses, we also removed any individuals that returned fewer than 2,000 loci.

Population genetics Principal Components Analysis

To visualize population genetic structure across all of our samples, we first ran a Principal Components Analysis (PCA). The PCA decomposes genetic variation across all samples and all SNPs into composite axes that explain the most variation in the dataset. We ran the PCA using *ipyrad*'s built-in analysis tool because of its ability to impute the values of missing SNPs to minimize their effect on the analysis. For the PCA, we only included one SNP per RAD locus, and only included SNPs present in more than 50% of individuals. We used the "sampled" method of imputation for missing data, which randomly samples genotypes based on the frequency of alleles across all samples. We visualized the results in R (R Core Team 2021) using the package *ggplot2* (Wickham 2016). In addition to PCA analyses for the full dataset, we repeated this analysis with individuals grouped by river drainage of origin using identical parameters as above for each subset of individuals.

Population genetic structure analyses

To quantify the number of populations that best explain our genetic data, we used a Bayesian clustering method called fastSTRUCTURE (Raj et al. 2014). Because structuring programs can be sensitive to missing data, we filtered our original VCF to include only SNPs present in more than 75% of individuals, and selected the single SNP with the highest coverage for each RAD locus (Pritchard et al. 2000; Newman and Austin 2016; Hodel et al. 2017). We ran fastSTRUCTURE for models of one (K = 1) through five (K = 5) populations, to account for more populations than river drainages (Apodaca et al. 2023). We used the built-in model evaluation tools in fastSTRUCTURE (chooseK.py) to evaluate which K value provided the best explanation of the data. In addition to structure analyses for the full dataset, we repeated the analyses with individuals grouped by river drainage of origin using identical parameters as above for each subset of individuals.

Genetic diversity, population subdivision, and effective population size analyses

For each of the populations identified by fastSTRUCTURE, and for each river drainage, we calculated a variety of statistics using R package *hierfstat* (Goudet 2005) using the same set of SNPs as in the fastSTRUCTURE analyses. To estimate genetic diversity, we calculated observed heterozygosity (H_O; Nei 1987) and within population gene diversity (i.e., expected heterozygosity, H_S; Nei 1987; Goudet 2005). To quantify levels of inbreeding, we calculated within-population subdivision (F_{IS}; Nei 1987). To measure population connectedness or differentiation, we calculated pairwise F_{ST} (Weir and Goudet 2017). We calculated effective population size (N_e) from the program NeEstimator (Do et al. 2014). This was calculated using the bias-corrected linkage disequilibrium method and we report N_e values resulting in no minor allele frequency cutoff (Waples and Do 2010). Using the same dataset, we also calculated these statistics separately for each for waterbody within a given basin, and calculated F_{ST} for each locality within a given basin.

Evaluation of the effect of dams on genetic connectivity
To assess the effect of dams on genetic connectivity, we compared population subdivision (F_{ST}) values above and below dams, where data were robust enough for comparisons, using the same dataset as in the fastSTRUCTURE analyses but sub-setting the dataset to just the individuals in the localities of interest. We explicitly tested whether F_{ST} values were higher across a dam than one would expect by chance, which would indicate further genetic divergence from side of the dam to the other. We examined locality pairs where the two sampling points both contained at least two individuals per locality, and where only one dam separated the two localities using a permutation test with $\alpha = 0.05$. We generated a null distribution of F_{ST} values by calculating F_{ST} for 1000 different permutations of the individuals in the original locality comparison. For example, at Lake Livingston, we sequenced individuals from Palmetto Creek (upstream of Lake Livingston) and individuals from Little Bayou (downstream of Lake Livingston). We then calculated F_{ST} for those individuals in 1000 random combinations and compared this modeled distribution of F_{ST} values to the actual F_{ST} value. If the actual F_{ST} value was higher than 95% of all permutations, we considered the dam to have increased F_{ST} over what is expected by chance.

Chromosome sex-linked marker discovery
Because ASTs can be difficult to reliably sex using external characteristics without a secondary sexing technique, we attempted to find markers on the W chromosome that might help distinguish males from females, though we knew that by chance we may never recover loci on the W chromosome given the stochastic nature of 3RAD. We used R package *radiator* (Gosselin 2020) to detect sex-linked markers and the function sexy_markers, which uses a random forest and machine learning approach to find markers unique to one sex. Because the sexy_markers function needs to find markers that segregate only because of sex, and not because of natural selection or population structure, we ran the program on the single population with the greatest number of successfully sexed individuals. We ran sexy_markers on the full number of SNPs in our dataset, as the program filters automatically for missing-ness and linkage disequilibrium.

Physiological data analyses

Morphometric data analyses
Because some individuals were recaptured throughout the course of the study, only measurements for the capture event resulting in the most complete set of morphometric values were used to reduce redundancy in data. Though some data were non-parametric, a two-way ANOVA was performed to detect differences in morphometric measurements for each sex by metapopulation. These analyses determined a significant difference between metapopulations, therefore, the data were split for subsequent analyses and re-evaluated using appropriate parametric and non-parametric tests (one-way ANOVA with pairwise comparisons using Holm-Sidak method or Kruskal-Wallis one-way ANOVA on ranks with Dunn's method for post-hoc comparisons, respectively) (Kruskal and Wallis 1952, Dunn 1961, Holm 1979).

In addition to morphometric data collected during field surveys, unpublished morphometric data from other agencies were provided for inclusion in analyses (Carl Franklin, Viviana Ricardez, and Sal Scibetta, Texas Turtles, Grapevine, Texas; Eric Munscher, Turtle Survival Alliance, Houston, Texas; Cindy Jones, Texas A&M University-Commerce, Commerce, Texas). We confirmed that measurements were collected using the same protocols as described above and excluded any measurements that were not consistently recorded prior to analyses. Available data for body size measurements from previous surveys were added to the overall morphometric dataset in order to compile the most robust morphometric data available for the life history of the species in Texas. Three previous surveys provided raw midline straight carapace length (mid-SCL) data (Nelson 1999, Rudolph et al. 2002, Riedle 2014). Only two previous surveys provided data for mass (Nelson 1999, Rudolph et al. 2002). For previous surveys where morphometric values were reported as means, standard errors, and ranges, we report those same values and did not include them in the larger morphometric analyses (Nelson 1999, Riedle 2014, Munscher et al. 2023). Some morphometric data from Munscher et al. (2020a) were also included in overall calculations presented in Munscher et al. (2023), therefore we do not report values from Munscher et al. (2020a).

Sonographic data analyses
Maximum follicle diameter and timing for quiescent females from sonographic analyses were plotted by observation date. For individuals with follicles observed in both sides of the body cavity, an average was calculated from the maximum follicle diameters of each side as long as these values were comparable (e.g., if the largest maximum follicle size on the left was > 0.1 cm larger than the largest follicle on the right, the larger of the two values was used for analyses). If follicles were only observed in one side of the body cavity, the overall maximum follicle diameter (regardless of side in the body cavity) was plotted. We estimated the periods and rates of follicular development and atresia based on a positive or negative, respectively, linear regression using these maximum follicle sizes.

Habitat associations

Small-scale habitat statistical analyses were conducted using R 2022.07.2 (RStudio Team 2021). The relationship between sites with status = 1 (AST detected) versus sites with status = 0 (no AST detected) were evaluated to determine the site characteristic(s) that maximized their detection and predicted occurrence using Kruskal-Wallis rank sum test with subsequent post-hoc Pairwise Wilcoxon rank sum test (Bauer 1972, Hollander et al. 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 AST would be detected at a site. Models were compared using Akaike Information Criterion (AIC). Additionally, non-metric multidimensional scaling (NMDS) plots and cluster analyses were conducted using PRIMER to assess relationships between AST and other species collected as bycatch.

Total of all observed counts for fishing gear were calculated by type due to our inability to precisely differentiate between active and derelict gear status (e.g., we did not evaluate anthropogenic influences based on gear status). Cumulative total of all fishing gear types was calculated for final analyses and categorized as “None”, “Low”, “Moderate”, and “High” using a Jenks Natural Breaks analysis to determine ranges for each category. These ranges differed from previous anthropogenic assessments which were determined after visual comparison of the data (David Rosenbaum, Stephen F. Austin University, *personal communication*; Rosenbaum et al. 2022). Similarly, counts of boat ramps and docks within the trapping area were combined due to variation in number, size, and use of structures. Statistical analyses of fishing gear and access point data were conducted using one-way ANOVA and linear regressions.

Site selection matrix data analyses

Scores from sites used in the current study were split into two groups: sites with positive AST detections and sites without AST detections. These groups were compared using Kruskal-Wallis one-way ANOVA on ranks and alpha values set to 0.05. Based on results from these group comparisons and likelihood of AST detection from field survey results, final cumulative scores were split into three prioritization categories: High priority, Moderate priority, and Low priority. To determine values limits for these categories, median score for sites with confirmed AST presence were used as the upper value for the moderate category median score for sites without AST captures were used as the lower value for the moderate category. Validity of these categories was further tested by applying the proposed categories to scores for all historic occurrences, regardless of quality of the associated spatial or temporal data, using a Kruskal-Wallis one-way ANOVA on ranks. Significant differences between the category mean values for all occurrences were confirmed *post-hoc* using Dunn's test.

RESULTS

Historic Data Compilation

Compilation of LEK and non-LEK sources for site selection

We compiled 357 historic accounts from 209 unique sources. Our literature review resulted in 31 sources (Table 2) of unique spatial (n = 29) and temporal (n = 60) data. Five sources provided specific coordinates, though most provided enough information to be reliably georeferenced. In some instances, reports overlapped between sources. Additionally, a review of Big Thicket National Park herpetological inventories included references to two previous surveys, but no new data were produced. Five sources used the now-outdated genus "*Macroclemys*" in reference to *M. temminckii*. Rudolph et al. (2002) reported spatial and temporal data for field surveys as well as data compiled from mail-in survey results. Data from field surveys were categorized as non-LEK (n = 13 spatial and 16 temporal) while data resulting from mail-in surveys were categorized as LEK (n = 14 spatial and 15 temporal). Four sources did not provide quantities of ASTs observed and, in general, most sources (n = 16) documented ≤ 3 captures from 1 or 2 locations.

Other sources of non-LEK data included VertNet (n = 27 spatial and temporal data points) and the TXNDD (n = 25 spatial and temporal data points). Of 32 accounts extracted from VertNet, seven contained GPS coordinates and two were georeferenced. We combined two reports due to duplication of date and location data. Supplemental data provided by the University of Texas Arlington Amphibian and Reptile Natural Diversity collection allowed us to pair an additional 18 VertNet accounts with specific coordinates. Of the 37 results produced by the TXNDD data query, we excluded two due to inadequate spatial and temporal data and four due to overlap with the VertNet data. Additionally, six resulted from a previous extraction of iNaturalist data performed by the TPWD. These reports were no longer accessible on iNaturalist and therefore excluded. Finally, two reports included sufficient spatial data, but did not provide specific temporal information.

Table 2 Results of literature review including reports of Alligator Snapping Turtle (AST; *Macrochelys temminckii*) spatial and/or temporal data from Texas. Literature types: JA = Journal Article; R = Report; B = Book; NHN = Natural History Note; GDN = Geographic Distribution Note; T = Thesis; D = Dissertation, PC = Public Comment. *N* = number of ASTs reported; Unk = unknown; NR = not reported.

Source	Topic	Source type	Survey area	*N*	Spatial resolution	Temporal resolution	Notes
Alhaboubi et al. (2017)	Clinical study	JA	Single location	1	City; county; state	Month; year	
Baxter-Bray et al. (2021)	Range extension	GDN	Single location	1	GPS coordinates; property; county; state	Day; month; year	
Ceballos and Fitzgerald (2004)	Turtle trade	JA	State-wide import/export	NR	State	None	Reported as *Macroclemys*
Crump (2010)	Species inventory	R	Two locations	2	Property; state	None	Same individuals in Nadeau et al. (2016)
Dixon (2013)	Field guide	B	State-wide	NR	County; state	None	
Echelle et al. (2010)[a]	Conservation genetics	JA	Range-wide (two locations in TX)	21	Waterbody; county; state	None	Same individuals in Roman et al. (1999)
Farr et al. (2005)	Basking	NHN	Single location	1	Property; state	Time; day; month; year	
Fisher and Rainwater (1978)	Species inventory	R	Single location	1	Property; state	None	Reported as *Macroclemys*; same individual in Nadeau et al. (2016)
Fitzgerald and Nelson (2011)[b]	Thermal biology	JA	Single location	16	GPS coordinates; waterbody; county; state	Day; month; year	Reported as *Macroclemys*; same individuals in Nelson (2011)
Franklin and Catalan (2009)	Range extension	GDN	Single location	1	Waterbody; road crossing; city; county; state	Day; month; year	
Franklin (2018)	Diet	NHN	Single location	1	Waterbody; county; state	Day; month; year	Report of stomach contents from dead *M. temminckii*
Franklin et al. (2018)	Defensive behavior	NHN	Single location	1	Waterbody; city; county; state	Time; day; month; year	
Franklin et al. (2020)	Caudal prehensility	NHN	Single location	1	Property; county; state	Time; day; month; year	
Franklin and Ricardez (2021)	Lenticular opacity	NHN	Single location	1	Waterbody; city; county; state	Time; day; month; year	
Franklin et al. (2021)	Diet	NHN	Single location	1	GPS coordinates; waterbody; city; state	Day; month; year	
Gordon et al. (2023)[c]	Local ecological knowledge	JA	Range-wide in Texas	245	Waterbody, county	Season, year	Includes spatial and temporal data from this report
Hay (1911)	Fossil specimen	JA	Single location	1	Waterbody; city; state	None	
Hibbitts and Hibbitts (2016)	Field guide	B	State-wide	NR	County; state	None	
Iverson and Hudson (2005)	Diet	NHN	Single location	1	Waterbody; road crossing; city; county; state	Day; month; year	
Kazmaier et al. (2010)[d]	Demography & habitat	R	Two locations	16	Property; state	Year range	Same individuals in Riedle (2014)

Table 2 Results of literature review including reports of Alligator Snapping Turtle (AST; *Macrochelys temminckii*) spatial and/or temporal data from Texas. Literature types: JA = Journal Article; R = Report; B = Book; NHN = Natural History Note; GDN = Geographic Distribution Note; T = Thesis; D = Dissertation, PC = Public Comment. *N* = number of ASTs reported; Unk = unknown; NR = not reported.

Source	Topic	Source type	Survey area	*N*	Spatial resolution	Temporal resolution	Notes
Munscher et al. (2019)	Range extension	GDN	Single location	1	GPS coordinates; waterbody; county; state	Day; month; year	
Munscher et al. (2020a)	Urban population	JA	Single location	57	Waterbody; county; state	Day; month; year	Includes individuals in Munscher et al. (2023)
Munscher et al. (2023)[c]	Population demographics	JA	Single location	155	Waterbody, city, state	Year	Includes some individuals from Munscher et al. (2020a)
Nadeau et al. (2016)	Resource assessment	R	Single location	3	Property; state	None	Includes individuals in Fisher and Rainwater (1978) and Crump (2010)
Nelson (1999)[b]	Thermal biology	T	Single location	66	Waterbody; county; state	Day; month; year	Reported as *Macroclemys*; includes individuals in Fitzgerald & Nelson (2011)
Norrid et al. (2021)	Range extension	GDN	Single location	1	GPS coordinates; waterbody; county; state	Day; month; year	Dead individual washed up on Gulf of Mexico side of beach
Pritchard (1989)	Biology & conservation	B	Range-wide (including TX)	NR	Waterbody; county; state	None	
Riedle (2014)[c]	Aquatic assemblages	D	Two locations	16	Property; county; state	Month; year	Includes individuals in Kazmaier et al. (2010) and Riedle et al. (2015, 2016)
Riedle et al. (2015)[d]	Aquatic assemblages	JA	Single location	12	Property; county; state	Month; year	Same individuals in Riedle (2014)
Riedle et al. (2016)[d]	Habitat associations	JA	Single location	12	Property; county; state	Month; year	Same individuals in Riedle (2014)
Roman et al. (1999)[d]	Population structure	JA	Range-wide (two locations in TX)	23	Basin; state	None	Includes individuals in Eschelle et al. (2010)
Rosenbaum (2022)[c,d]	Population assessment	T	Range-wide in Texas	Unk	Unknown	Unknown	Includes individuals in Rosenbaum et al. (2022, 2023)
Rosenbaum et al. (2022)[c,d]	Population assessment	R	Range-wide in Texas	214	Waterbody; county; basin; state	Year range	Includes individuals in Rosenbaum et al. (2023) and Rosenbaum (2022)
Rosenbaum et al. (2023)[c,d]	Population assessment	JA	Range-wide in Texas	Unk	Unknown	Unknown	Includes individuals in Rosenbaum et al. (2022) and Rosenbaum (2022)
Rudolph et al. (2002)	Population assessment	R	State-wide (16 locations in TX)	48	Waterbody; county; state	Day; month; year	Reported as *Macroclemys*; includes data for field surveys (non-LEK) & mail-in survey results (LEK)
USFWS (2021)[c]	Species status assessment	SSA	Range-wide in US	Unk	Region; map unit	All-time	

[a]Same individuals reported by both papers
[b]Same individuals reported in thesis and article; waterbody name in thesis was incorrect
[c]Data not used in spatial distribution for site selection process (published after selection occurred)
[d]Same individuals reported in report, thesis/dissertation, and papers

Sources of LEK included iNaturalist, the LNVA Mapper, social media, and personal communications. Of 150 accounts extracted from iNaturalist, 96 represented unique reports. We excluded four due to overlap with other sources where the observer provided more specific information (e.g., personal communication, LNVA Mapper, social media). Another four were excluded due to replication from an existing publication. We excluded 28 reports due to their relationship with ongoing assessments. Remaining reports included specific temporal data for analyses (n = 60). Due to spatial obscuring, we used 11 reports (18.3%) in spatial analyses after users provided additional information. From January–October 2021, 48 reports were made to the LNVA Mapper. Of these, we excluded 6 for various reasons (location outside of Texas: n = 3, overlap with ongoing studies: n = 1, overlap with other data source type: n = 1, lack of sufficient data: n = 1). The remaining 42 contained accurate spatial and temporal data for analyses. Social media resulted in 18 reports with useful spatial data and 21 with temporal data. Between January–July 2021, 18 personal communications resulted in 47 reports of AST. For 44 of these reports, individuals provided spatial data. While many were not able to provide specific dates (< 43%), most were able to provide sufficient season or year data for temporal analyses (n = 39).

Spatial data compiled for site selection

Across all historic accounts, 196 unique spatial datapoints were compiled (non-LEK: n = 67; LEK: n = 129) (Figure 13). Sites selected based on non-LEK (n = 4) and LEK (n = 21) had similar detection success (75.0% and 71.4%, respectively) while sites selected based on habitat alone (n = 16) had lower detection success (31.3%) (Table 3). In instances where more than one selection criteria were used (e.g., LEK and habitat, or non-LEK and habitat; n = 7), we saw similar detection success to when LEK or non-LEK reports were used alone (71.4%).

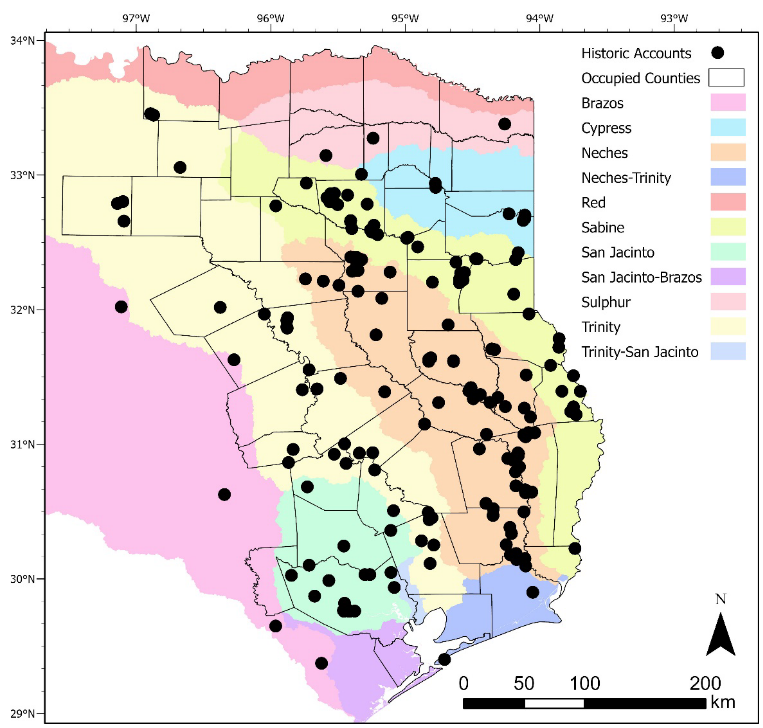


Figure 13 Final distribution of historic accounts (n = 196) used to guide site selection for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in east Texas. Historic accounts were compiled from peer-reviewed publications, agency reports, VertNet, the Texas Natural Diversity Dataset (TXNDD), iNaturalist, the Lower Neches Valley Authority (LNVA) Mapper, social media, and personal communications. Historic accounts from previous or ongoing studies have been removed. Larger point locations represent overlap of historic accounts.

Table 3 Sites selected for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) trapping surveys based on historic observations (LEK = local ecological knowledge, Non-LEK = previously published literature or databases, habitat = aerial imagery from Google Earth and site selection matrix). Numbers in parentheses indicate number of sources for each selection criterion type. Year of last occupancy based on historic range presented in *M. temminckii* Species Status Assessment (USFWS 2021). Counties not in historic range indicated as "NA".

Basin	Waterbody name	County	Selection criteria	Year of last occupancy	AST detected
Brazos	South Bosque River	McLennan	Habitat	NA	No
	Scoby Lake	Brazoria	LEK (1)	NA	No
	Brazos River	Fort Bend	Habitat	NA	No
Cypress	Kitchen Creek	Marion	LEK (1)	2009	No
	Caddo Lake	Harrison	LEK (1)	2015	No
	Big Cypress Bayou	Marion	LEK (1)	2009	Yes
Neches	Pine Island Bayou	Jefferson / Hardin	LEK (3)	2013 / 2018	Yes
	Pinkston Reservoir	Shelby	Non-LEK (1), LEK (1)	2016	Yes
	Big Sandy Creek	Polk	LEK (1)	2013	Yes
	Angelina River	Jasper	LEK (2)	2016	Yes
Neches-Trinity	East Fork Double Bayou	Chambers	Habitat	Unknown	No
	South Fork Taylor Bayou	Jefferson	Habitat	NA	No
	Hillebrandt Bayou	Jefferson	LEK (1), Habitat	NA	No
Red	Craddock Creek	Lamar	LEK (2), Habitat	NA	Yes
	McKinney Bayou	Bowie	Non-LEK (1), LEK (1)	2010	Yes
	Big Pine Creek	Red River	LEK (1)	2014	Yes
Sabine	Martin Lake	Rusk	Non-LEK (10)	2016	No
	Big Cow Bayou	Newton	LEK (1)	2000	Yes
	Swift Slough/Sabine River	Orange	LEK (1), Habitat	2013	Yes
	South Fork Sabine River	Rains / Hunt	Habitat	1985 / NA	Yes
San Jacinto	Cypress Creek/Marshall Lake	Harris	LEK (1)	2019	Yes
	East Fork San Jacinto	Liberty	LEK (1)	2016	Yes
	Luce Bayou	Harris	LEK (1)	2019	Yes
	Spring Creek	Harris	Non-LEK (1), LEK (2)	2019	Yes
San Jacinto-Brazos	Chocolate Bayou	Brazoria	Habitat	NA	No
	Lemon Reservoir/Austin Bayou	Brazoria	Habitat	NA	No
Sulphur	Wright Patman Lake/Milan Creek	Bowie	Habitat	NA	No
	White Oak Creek	Morris	Habitat	NA	No
	South Sulphur River	Hopkins	LEK (1), Habitat	NA	No
Trinity	Palmetto Creek	San Jacinto	LEK (1)	2000	Yes
	Black Slough	Anderson	Habitat	2014	No
	Turtle Bayou	Chambers	Habitat	Unknown	Yes
	Little Bayou	Liberty	Habitat	2016	Yes
	Buck Creek	Grayson	LEK (1)	1993	No

Number of reports, number of AST reported, and CPUE

Across all historic sources, 274 datapoints contained temporal data (non-LEK: n = 97; LEK: n = 177) (Figure 14). While non-LEK sources provided more records in the > 10 years category, LEK provided more observations in the 4-9 and < 3 years categories (H = 108.000, $p < 0.001$). Though non-LEK resulted in more reports across all seasons, significantly more occurred in spring (H = 108.000, $p < 0.001$) and summer (H = 100.000, $p < 0.001$). Due to vagaries in description or an individual's inability to recall a specific date or month of an observation, 18 and 21 seasonal data points fell into an "unknown" category for non-LEK and LEK, respectively. Reports with reliable temporal data resulted in observations of 725 AST (n = 515 and 210 for non-LEK and LEK, respectively). Though more AST were documented from non-LEK sources across all year categories, no significant differences were detected. Non-LEK sources provided significantly more observations of AST when season was unknown (H = 7.094, $p = 0.008$), but when unknown season was excluded, no differences were detected between non-LEK and LEK by season ($F_{2,229} = 1.456$, $p = 0.235$). As with a number of reports, overall number of AST reported by both source types were highest in spring and summer (n = 140 and 147, respectively).

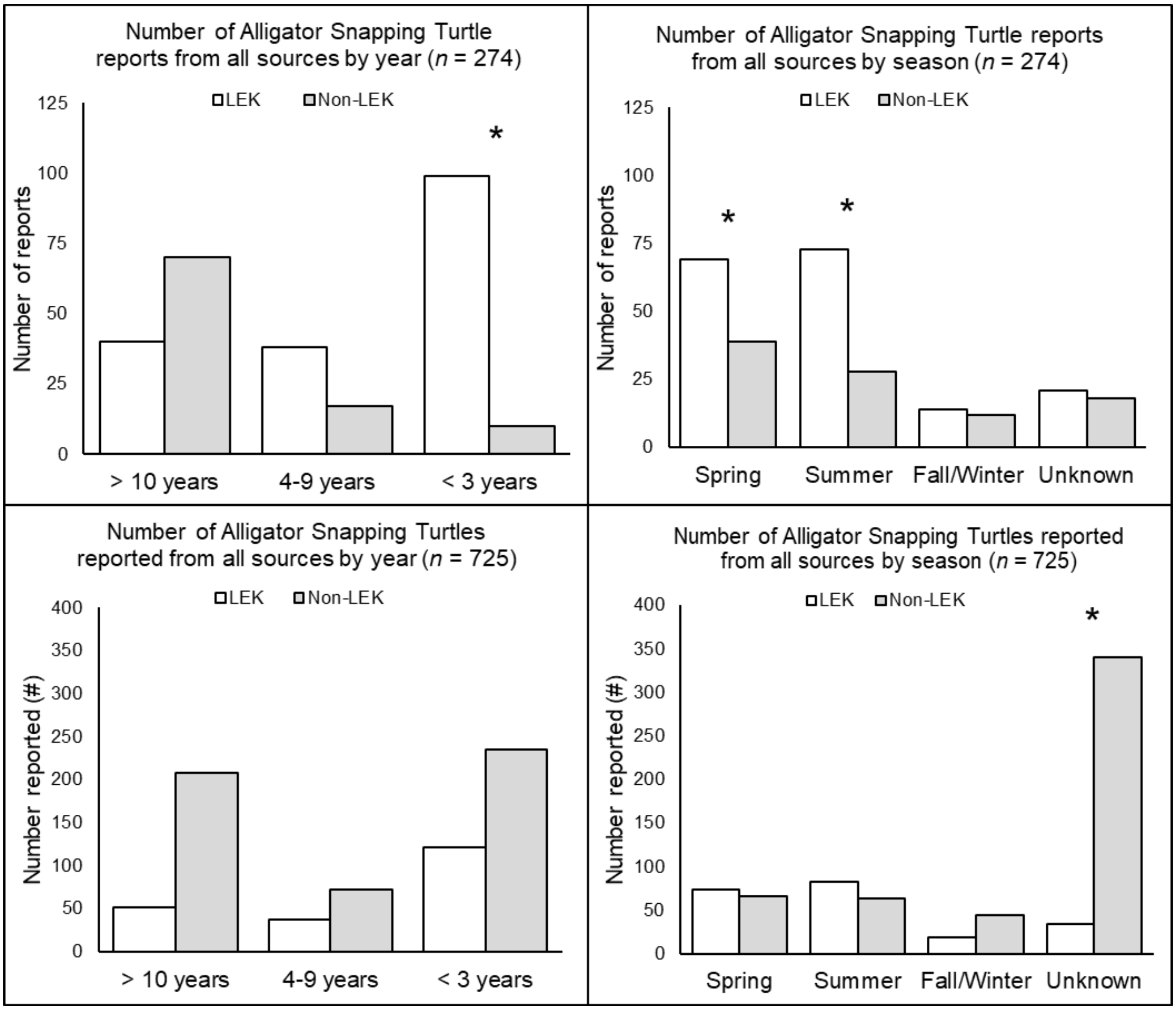


Figure 14 Alligator Snapping Turtle (AST; *Macrochelys temminckii*) reports from local ecological knowledge (LEK) and non-LEK derived data. *Top left*: number of AST reports from all sources by year groupings (n = 274). *Top right*: number of AST reports from all sources by season (n = 274). *Bottom left*: number of AST reported from all sources by year groupings (n = 725). *Bottom right*: number of AST reported from all sources by season (n = 725). Asterisks above bars represent categories with significant differences between LEK and non-LEK data.

Six previous assessments in Texas provided catch and CPUE data (Nelson 1999; Rudolph et al. 2002; Riedle 2014; Munscher et al. 2020a, 2023; Rosenbaum et al. 2022). Riedle (2014) documented number of ASTs captured and effort (number of trap nights) for two sites across a range of dates, so we calculated CPUE for each site and assigned season as "unknown". Similarly, Nelson (1999) and Rosenbaum et al. (2022) reported total capture number and overall CPUE across a range of dates so we were unable to accurately break down values by season and assigned season as "unknown". Munscher et al. (2023) reported total counts and CPUE by year from 2016-2021. Capture data from 2016-2018 had previously been published at a higher resolution (Munscher et al. 2020a), therefore we excluded data for these years in order to reduce redundancy. We excluded instances where season was unknown for seasonal comparisons. Overall, CPUE did not differ between historic surveys or the current survey ($p = 0.255$) (Figure 15). However, we detected an interaction between survey type and season ($F_{2,57} = 3.582$, $p = 0.034$), with historic CPUE rates being higher in the fall/winter season than in the current study.

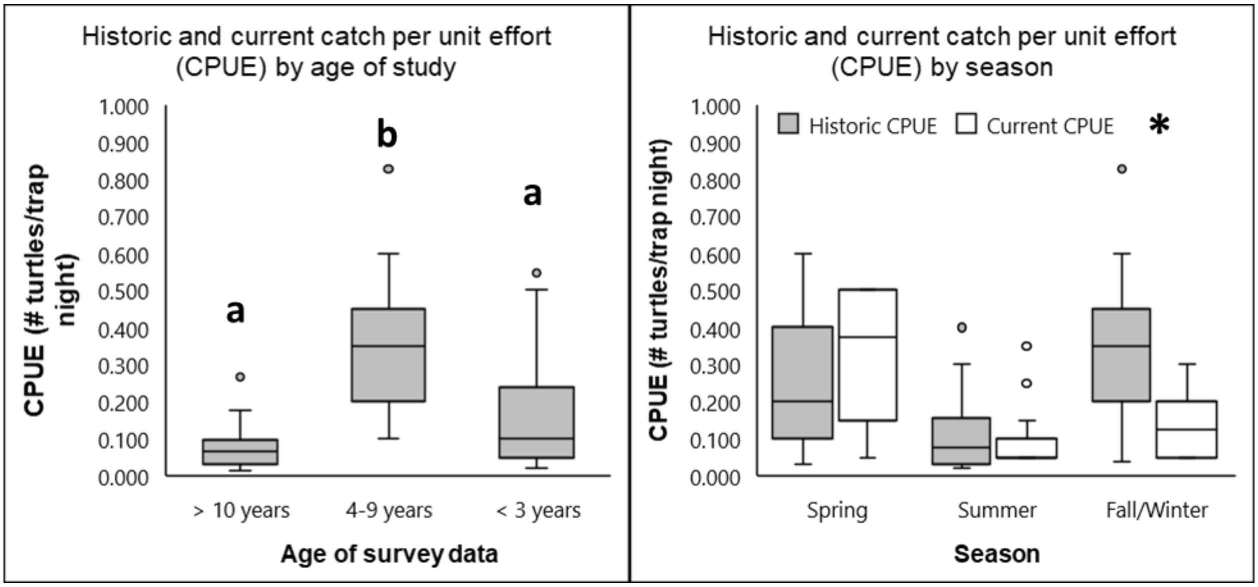


Figure 15 Catch per unit effort (CPUE; number of turtles per trap night) for current and historic Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in Texas by age of study (left; > 10 years ago, 4-9 years ago, or < 3 years ago) and by season (right; spring = February through May, summer = June through September, and fall/winter = October through January). Letters or asterisks above boxes represent significant groupings or interactions, respectively. Overall, CPUE did not differ by survey type (historic versus current; $p = 0.255$), but CPUE was significantly higher when reported 4-9 years ago ($H = 27.754$, df = 2, $p < 0.001$). We detected an interaction between survey type (historic versus current) and season, with CPUE during the fall/winter season with CPUE for historic surveys being greater than in the current study ($F_{2,57} = 3.582$, $p = 0.034$). All CPUE data were compiled from the current study, Nelson (1999), Rudolph et. al (2002), Riedle (2014), Munscher et al. (2020a, 2023), and Rosenbaum et al. (2022).

Age-size class matrix development and testing

We conducted a literature review to determine sizes at which individual AST were reported as “hatchlings”, “juveniles”, “sub-adults”, and “adults” (Dobie 1971, Pritchard 1989, Riedle et al. 2008, East et al. 2013, Ligon et al. 2014, Trauth et al. 2016, Huntzinger et al. 2019, Munscher et al. 2023). Though we found many inconsistencies in the literature and a marked lack of secondary sexing technique(s) beyond use of external physical characteristics, we were able to develop the proposed age-size class distributions outlined in Table 4.

Morphometric data (mid-SCL) for one female captured by Riedle (2014) were excluded from comparative analyses between the current and previous surveys as this was the only female caught in the survey and a mean could not be calculated. Using data for 118 individuals where we had paired data for age class assigned in the field and using the matrix, we detected significant differences in mid-SCL between adults ($n = 81$; mean = 444.2 mm mid-SCL), sub-adults ($n = 13$; mean = 334.5 mm), and juveniles ($n = 24$; mean = 240.3 mm) ($F_{2,115} = 88.312$, $p < 0.001$) when age-class was assigned in the field. We then compared age classes assigned using the matrix and confirmed significant differences between adults (median = 426 mm), sub-adults (median = 285 mm), and juveniles (median = 218 mm) ($H = 66.362$, df = 2, $p < 0.001$). When age class observed in the field did not match that assigned by the matrix, individuals were significantly smaller (mid-SCL = 316 mm) than when age class assignments matched (mid-SCL = 420 mm) (H = 19.411, df = 1, $p < 0.001$).

We were able to obtain corresponding mid-SCL and mass values for 245 individuals between previous studies and the current study. When all data were pooled, we detected interactions between data type (current versus historic) and sex ($p = 0.017$) and data type and metapopulation ($p < 0.001$). While we received morphometric measurements for 29 AST hatchlings from a single nest, when we excluded the hatchling age class, no significant interactions between data type (historic versus current), sex, or age class were detected. While we did detect an interaction between data type and metapopulation ($p = 0.045$), we attributed this difference to large variation in sample size (for example, 15 individuals in the Red-Cypress-Sulphur basin metapopulation versus 101 individuals in the San Jacinto-Trinity metapopulation; metapopulation results to follow in subsequent sections), so we proceeded with BCI, mid-SCL, and mass comparisons by pooling all remaining datapoints ($n = 215$).

Table 4 Criteria used for development of the proposed age and size class structure for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Classes were grouped based on examples from existing literature and tested using data collected during the current study. Size class is presented as a range based on midline straight carapace length (mid-SCL).

Age class	Size class (by mid-SCL)	Citations used for justification of size classes
Hatchling	< 45 mm	Sex unable to be determined for 2-month old hatchlings using laparoscopy (Ligon et al. 2014).
Juvenile	45 mm-249 mm	Minimum size at which 2-month old hatchlings ($n = 8$; mean mid-SCL = 44 mm ± 1) could be sexed using laparoscopy (Ligon et al. 2014). Sex determined for some individuals using external characteristics in 151-200 mm (males) and 251-300 mm (females) groups, but unknowns were not reported (Trauth et al. 2016); in same study, majority of males and females occurred within > 350 mm size group, though no secondary sexing technique was used. Other studies report differentiation between sexes in classes of 241-260 mm (Riedle et al. 2008) and 251-300 mm (Munscher et al. 2023).
Sub-adult	250 mm-320 mm	Sex determined in some individuals in 241-260 mm size class using external characteristics (East et al. 2013); external sex characteristics observed in some individuals > 295mm and sex confirmed by laparoscopy (Ligon et al. 2014).
Adult	> 320 mm	Fully mature adults noted using observations of external characteristics at > 295 mm (Ligon et al. 2014), > 330 mm (Pritchard 1989; Dobie 1971, females), > 320 mm (East et al. 2013), > 320 mm (Hilzinger et al. 2019), and > 370 mm midline straight carapace length (Dobie 1971, males), though no secondary sexing technique was used by any study.

To further test application of the proposed age-size class matrix, we compared BCI between age classes determined in the field and those assigned based on the matrix. Amongst the full dataset, mass and mid-SCL were strongly correlated ($R^2 = 0.9855$) (Figure 16). When comparing BCI among age classes for females and unknowns, significant differences were detected between age classes, regardless of assignment type (e.g., in the field or using the size-age class structure matrix; females: $p = 0.022$ and < 0.001, respectively; unknowns: $p = 0.001$ and < 0.001, respectively) (Figure 17). For males, we were unable to compare BCI among age classes assigned in the field due to low sample size (juvenile and sub-adult $n = 1$, respectively), but, when comparing male BCI among age classes assigned using the matrix, significant differences were detected ($p < 0.001$). In all cases, p-values were lower when values were compared against age class assigned by the matrix.

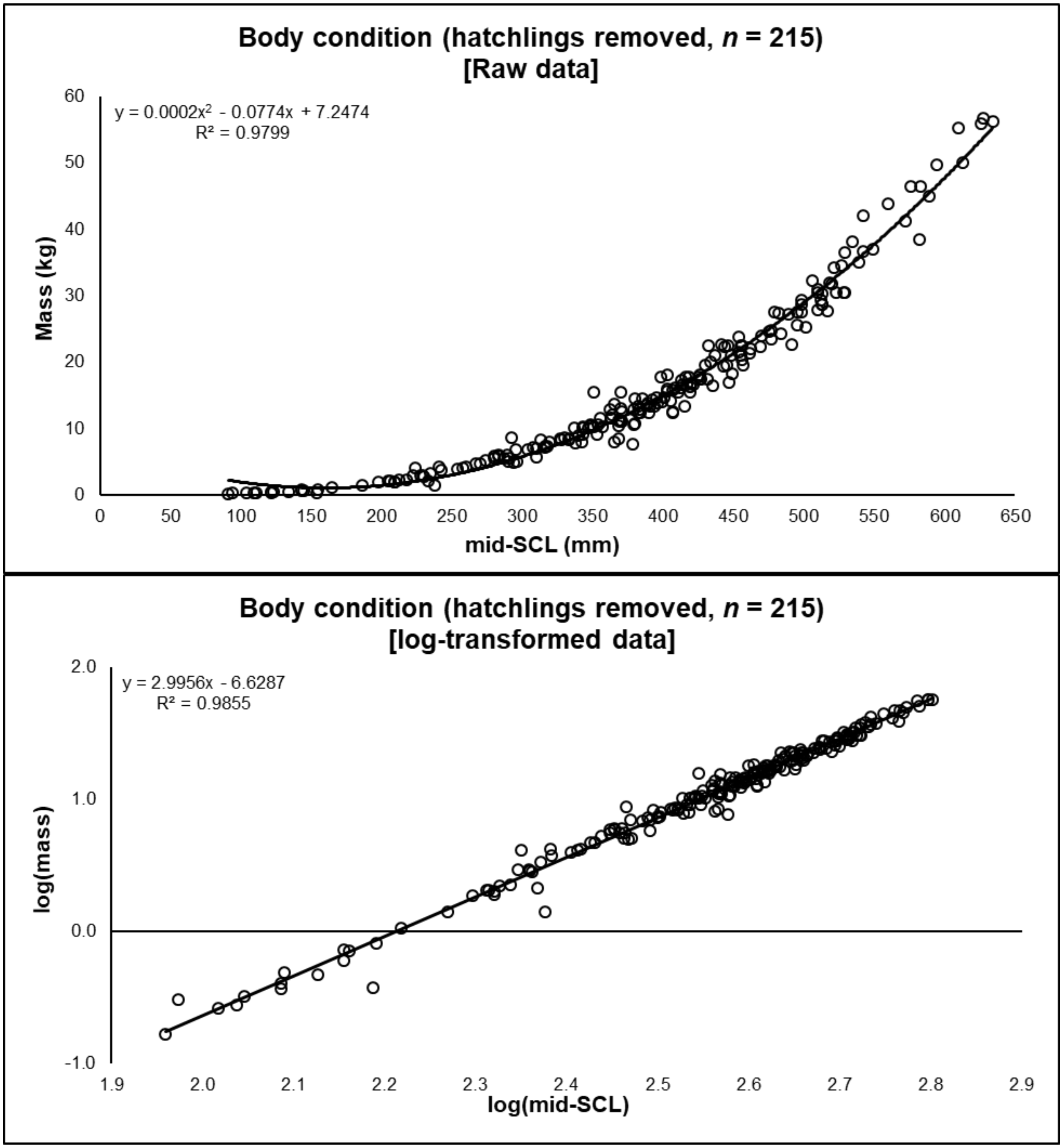


Figure 16 Body condition index (BCI) for Alligator Snapping Turtles (AST; *Macrochelys temminckii*) captured in the current and previous surveys with hatchlings (midline straight carapace length [mid-SCL] < 45 mm) excluded (n = 215). Body mass was strongly correlated with mid-SCL for raw ($R^2 = 0.9799$, $p < 0.001$) and log-transformed datasets ($R^2 = 0.9722$, $p < 0.001$).

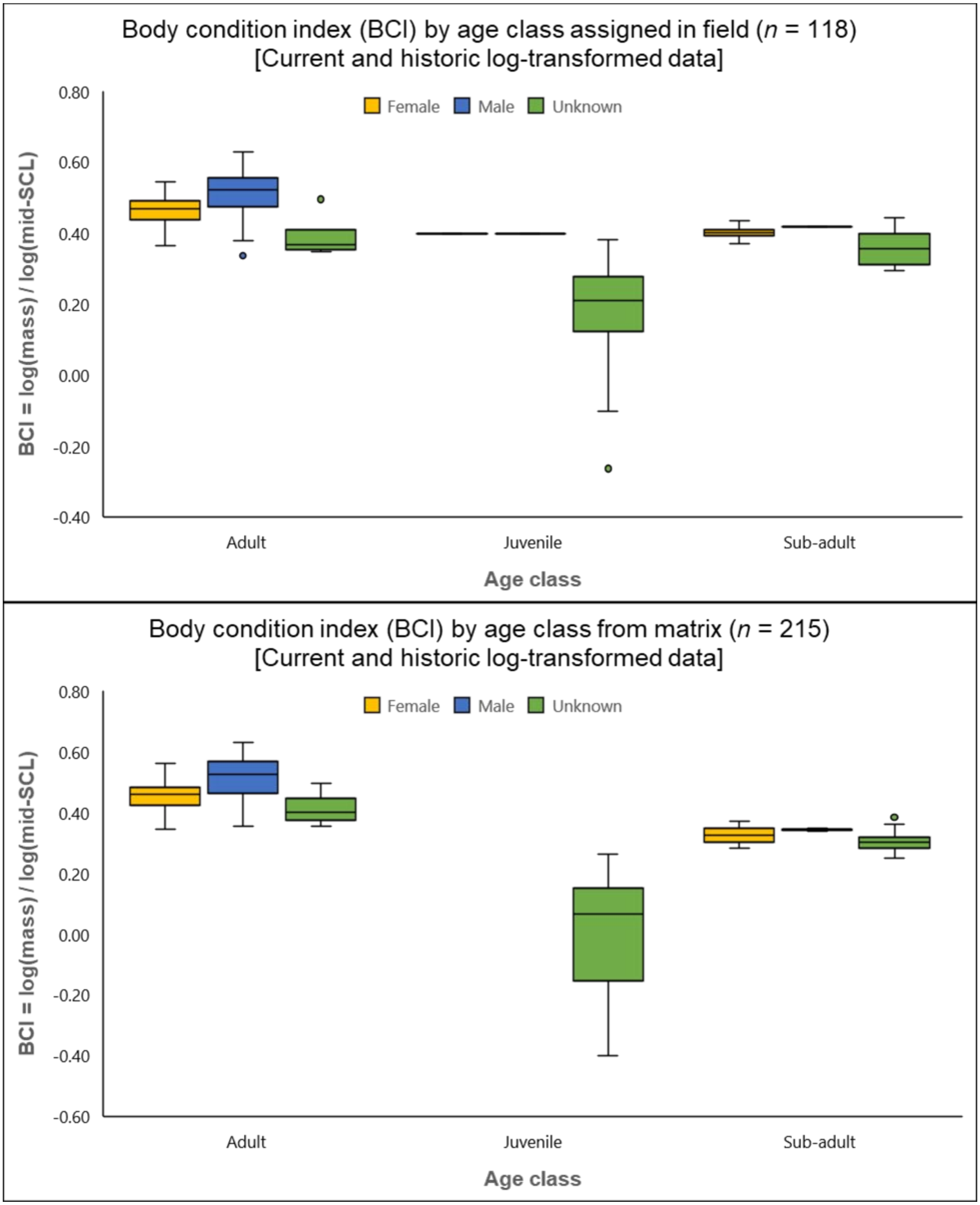


Figure 17 Body condition index (BCI) by sex for individual Alligator Snapping Turtles (AST; Macrochelys temminckii) documented from previous surveys (Nelson 1999, Rudolph et al. 2002, Fitzgerald and Nelson 2011) and the current survey. For females and unknowns, BCI was significantly different between age classes regardless of assignment type (e.g., in the field [top image] or calculated from the proposed age-size class matrix [bottom image]) (females: $p = 0.022$ and < 0.001, respectively; unknowns: $p = 0.001$ and < 0.001, respectively). For males, we were unable to compare BCI among age classes assigned in the field but, when comparing among age classes assigned using the matrix, significant differences were detected ($p < 0.001$).

Overall Site Distribution, Sampling Effort, CPUE, and Density Estimates

We conducted 83 surveys in 34 locations representing 25 counties. Overall, we captured AST at 24 (70.6%) sites (Figure 18). Sites were sampled from April 2021–November 2022 with 20 events occurring in the Spring season, 41 events in the Summer, and 22 events in the Fall/Winter (Table 5). Average trapping effort for each event was 18.8 ± 0.33 trap-nights (range: 8-20) and average length of the survey reach for all sites was 1,341.9 ± 72.35 river-kilometers (r-km) (range: 378-4,895) (Table 6). Overall, we trapped in 10 of the 11 east Texas river basins, as defined by the TWDB (2021). We were unable to conduct surveys in the San Jacinto-Trinity basin due to lack of available habitat and access permissions. Riverine habitat comprised 29 (85.3%) of the total sites while lacustrine habitat comprised five (14.7%) sites. Riverine sites had an overall higher proportion of detections than lacustrine (58.6% and 20%, respectively). Throughout the study, 78 captures representing 69 unique individuals were documented over 1,558 trapping nights. Average CPUE for all sampling events was 0.053 ± 0.0116 (range: 0.00–0.50) while CPUE for only events when ASTs were captured (n = 32) was 0.137 ± 0.0234 (range: 0.05–0.50). Across all sites, AST density averaged 0.772 turtles per r-km (range: 0.00-6.06), but at sites occupied by AST, density averaged 2.003 turtles per r-km (range: 0.43–6.06).

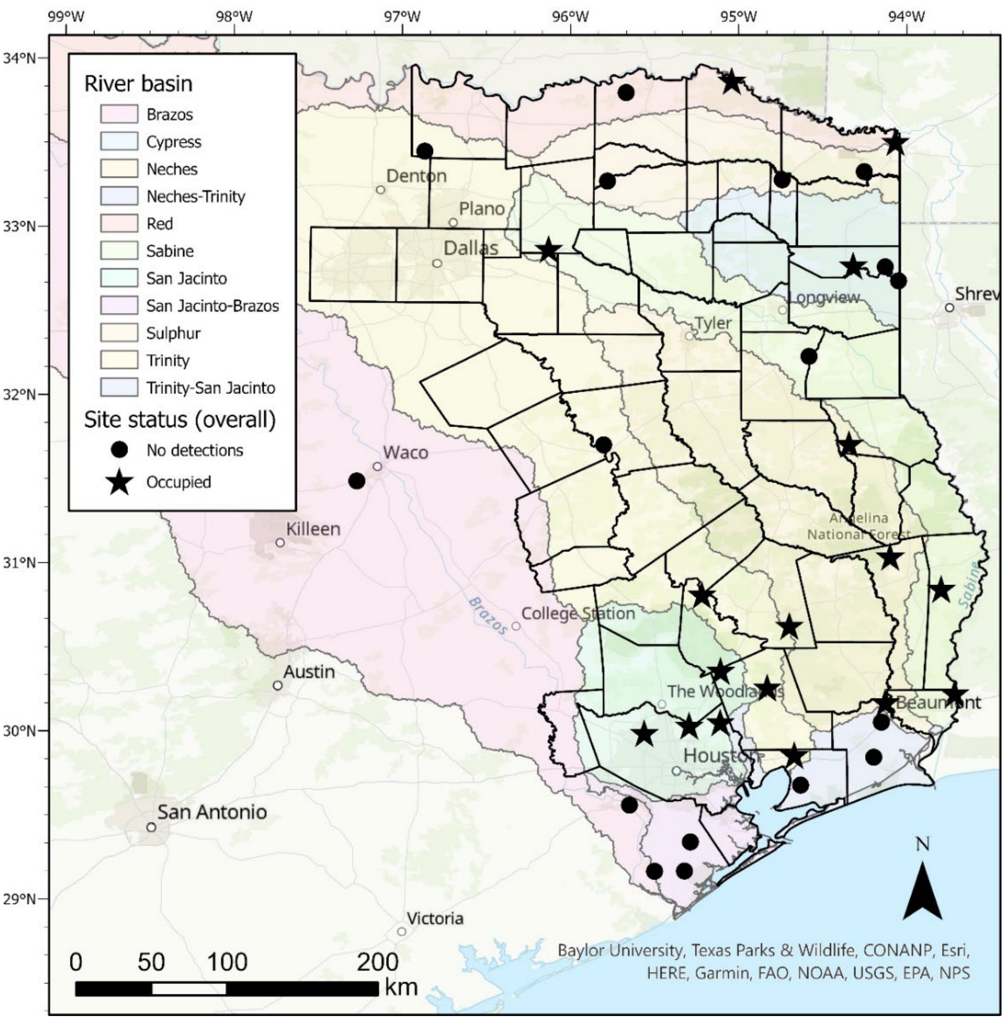


Figure 18 Sites sampled (n = 34) during Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in east Texas from March 2021 through November 2022. Sites where AST were captured are indicated with a star (n = 24) and sites where AST were not captured indicated with a black dot (n = 10).

Table 5 Sites sampled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from April 2021–November 2022. Includes waterbody type ("Wb type"), surrounding habitat type, overall occupancy status as determined by the current study ("Status"), recommended site designation for future assessments ("Recommended type"; primary = best recommendation; secondary = second best; exploratory and undetermined = not recommended), and metapopulation as determined from population genetics results ("Meta"; NA = not applicable; RCS = Red+Cypress+Sulphur basins, SN = Sabine+Neches basins, ST = San Jacinto+Trinity basins).

Basin	Site ID	Waterbody name	County	Wb type	Surrounding habitat	Status	Recommended type	Meta
Brazos	BRB-02	South Bosque River	McLennan	Riverine	Forest	No detections	Exploratory	NA
	BRB-06	Scoby Lake	Brazoria	Lake	Forest	No detections	Exploratory	NA
	BRB-10	Brazos River	Fort Bend	Riverine	Park	No detections	Exploratory	NA
Cypress	CRB-04	Kitchen Creek	Harrison/Marion	Lake	Forest	No detections	Undetermined	RCS
	CRB-09	Caddo Lake	Harrison	Lake	Forest	No detections	Undetermined	RCS
	CRB-10	Big Cypress Bayou	Marion	Riverine	Forest	Occupied	Primary	RCS
Neches	NRB-04	Pine Island Bayou/Cooks Lake	Hardin/Jefferson	Riverine	Forest	Occupied	Secondary	SN
	NRB-07	Pinkston Reservoir	Shelby	Lake	Forest	Occupied	Secondary	SN
	NRB-08	Big Sandy Creek	Polk	Riverine	Forest	Occupied	Primary	SN
	NRB-09	Old Angelina Corridor	Jasper	Riverine	Forest	Occupied	Secondary	SN
Neches-Trinity	NTB-03	East Fork Double Bayou	Chambers	Riverine	Forest	No detections	Exploratory	NA
	NTB-06	South Fork Taylor Bayou	Jefferson	Riverine	Forest	No detections	Exploratory	NA
	NTB-11	Oxbow of Hillebrandt Bayou	Jefferson	Riverine	Rural/Pasture	No detections	Exploratory	NA
Red	RRB-01	Craddock Creek	Lamar	Riverine	Forest	No detections	Undetermined	RCS
	RRB-05	McKinney Bayou	Bowie	Riverine	Rural/Pasture	Occupied	Secondary	RCS
	RRB-06	Big Pine Creek	Red River	Riverine	Forest	Occupied	Undetermined	RCS
Sabine	SAB-02	Martin Lake	Shelby	Lake	Forest	No detections	Undetermined	SN
	SAB-04	Big Cow Creek	Newton	Riverine	Rural/Pasture	Occupied	Secondary	SN
	SAB-05	Sabine River/Swift Bayou	Orange	Riverine	Forest	Occupied	Secondary	SN
	SAB-06	South Fork Sabine River	Hunt	Riverine	Forest	Occupied	Secondary	SN
San Jacinto	SAJ-01	Cypress Creek	Harris	Riverine	Park	Occupied	Primary	ST
	SAJ-03	East Fork San Jacinto	Liberty	Riverine	Forest	Occupied	Secondary	ST
	SAJ-04	Luce Bayou	Harris	Riverine	Forest	Occupied	Secondary	ST
	SAJ-05	Spring Creek	Harris	Riverine	Park	Occupied	Primary	ST
San Jacinto-Brazos	SJB-03	Chocolate Bayou	Brazoria	Riverine	Park	No detections	Exploratory	NA
	SJB-05	Austin Bayou/Lemon Reservoir	Brazoria	Riverine	Rural/Pasture	No detections	Exploratory	NA
Sulphur	SUB-01	Milan Creek	Bowie	Riverine	Forest	No detections	Undetermined	RCS
	SUB-02	White Oak Creek	Morris	Riverine	Forest	No detections	Undetermined	RCS
	SUB-05	South Sulphur River	Hopkins	Riverine	Forest	No detections	Exploratory	RCS
Trinity	TRB-01	Palmetto Creek	San Jacinto	Riverine	Forest	Occupied	Primary	ST
	TRB-02	Black Slough	Anderson	Riverine	Forest	No detections	Undetermined	ST
	TRB-04	Turtle Bayou	Chambers	Riverine	Forest	Occupied	Secondary	ST
	TRB-05	Little Bayou	Liberty	Riverine	Forest	Occupied	Secondary	ST
	TRB-16	Buck Creek	Grayson	Riverine	Forest	No detections	Undetermined	ST

Table 6 Survey sites, season sampled, survey date(s), total reach length in river kilometers (r-km), effort in number of trap nights, total number of Alligator Snapping Turtles (AST; *Macrochelys temminckii*), density of AST as number of turtles per r-km, and catch per unit effort (CPUE, number of turtles per trap night) for each survey event conducted in the current study. Surveys were conducted from April 2021–November 2022. Asterisks (*) indicate events that were terminated early due to local conditions.

Site ID	Season	Survey dates	Reach length (r-km)	Effort	Total # AST	AST Density	AST CPUE
BRB-02	Fall/winter	10/12/2021-10/13/2021*	1,752	10	0	0.000	0.000
	Summer	08/30/2022-09/01/2022	508	18	0	0.000	0.000
BRB-06	Summer	06/07/2021-06/09/2021	941	20	0	0.000	0.000
	Fall/winter	09/30/2021-10/02/2021	1,390	20	0	0.000	0.000
BRB-10	Spring	04/26/2022-04/28/2022	1,034	20	0	0.000	0.000
	Summer	08/02/2022-08/04/2022	1,357	20	0	0.000	0.000
CRB-04	Fall/winter	10/05/2021-10/07/2021	584	20	0	0.000	0.000
CRB-09	Fall/winter	10/05/2021-10/07/2021	556	18	0	0.000	0.000
CRB-10	Fall/winter	10/26/2021-10/28/2021	2,381	20	3	1.260	0.150
	Spring	05/20/2022-05/22/2022	1,378	20	0	0.000	0.000
	Summer	08/30/2022-09/01/2022	2,293	20	1	0.436	0.050
	Fall/winter	11/08/2022-11/10/2022	1,359	20	0	0.000	0.000
NRB-04	Summer	09/07/2021-09/09/2021	2,190	20	1	0.457	0.050
	Spring	03/01/2022-03/03/2022	2,541	20	0	0.000	0.000
	Summer	08/25/2022-08/27/2022	1,964	20	1	0.509	0.050
NRB-07	Summer	06/01/2021-06/03/2021	1,871	20	1	0.534	0.050
	Spring	03/23/2022-03/25/2022	1,876	20	0	0.000	0.000
	Summer	09/06/2022-09/08/2022	1,119	20	0	0.000	0.000
NRB-08	Summer	08/03/2021-08/05/2021	1,416	20	7	4.944	0.350
	Fall/winter	12/14/2021-12/16/2021	936	20	1	1.068	0.050
	Spring	05/02/2022-05/04/2022	876	20	1	1.142	0.050
	Summer	07/11/2022-07/13/2022	735	20	0	0.000	0.000
	Fall/winter	10/13/2022-10/15/2022	718	20	2	2.786	0.100
NRB-09	Summer	07/26/2021-07/28/2021	1,334	20	1	0.750	0.050
	Spring	03/29/2022-03/31/2022	2,053	20	0	0.000	0.000
	Summer	06/21/2022-06/22/2022*	1,463	10	0	0.000	0.000
NTB-03	Summer	08/16/2021-08/18/2021	1,925	20	0	0.000	0.000
	Spring	03/15/2022-03/17/2022	1,362	20	0	0.000	0.000
NTB-06	Summer	05/31/2022-06/02/2022	840	20	0	0.000	0.000
	Fall/winter	10/31/2022-11/02/2022	978	20	0	0.000	0.000
NTB-11	Spring	04/12/2022-04/14/2022	906	20	0	0.000	0.000
	Summer	08/01/2022-08/03/2022	584	20	0	0.000	0.000
RRB-01	Summer	09/20/2022-09/22/2022	884	20	1	1.131	0.050
RRB-05	Fall/winter	11/30/2021-12/02/2021	378	18	0	0.000	0.00^

Table 6 Survey sites, season sampled, survey date(s), total reach length in river kilometers (r-km), effort in number of trap nights, total number of Alligator Snapping Turtles (AST; *Macrochelys temminckii*), density of AST as number of turtles per r-km, and catch per unit effort (CPUE, number of turtles per trap night) for each survey event conducted in the current study. Surveys were conducted from April 2021–November 2022. Asterisks (*) indicate events that were terminated early due to local conditions.

Site ID	Season	Survey dates	Reach length (r-km)	Effort	Total # AST	AST Density	AST CPUE
	Summer	07/07/2022-07/09/2022	779	16	1	1.284	0.063
	Fall/winter	10/10/2022-10/12/2022	944	20	0	0.000	0.000
RRB-06	Summer	09/23/2022-09/25/2022	1,172	20	2	1.706	0.100
SAB-02	Summer	06/15/2021-06/17/2021	4,895	20	0	0.000	0.000
SAB-04	Spring	05/25/2021-05/26/2021*	853	10	0	0.000	0.000
	Summer	08/27/2021-08/29/2021	783	20	1	1.277	0.050
	Summer	08/08/2022-08/10/2022	872	18	0	0.000	0.000
	Fall/winter	11/16/2022-11/18/2022	938	20	0	0.000	0.000
SAB-05	Summer	07/21/2021-07/23/2021	1,193	18	1	0.838	0.056
	Spring	02/15/2022-02/16/2022*	1,240	10	0	0.000	0.000
	Summer	08/15/2022-08/17/2022	1,133	20	0	0.000	0.000
SAB-06	Summer	07/13/2021-07/15/2021	1,237	20	1	0.808	0.050
	Spring	05/17/2022-05/19/2022	1,034	20	0	0.000	0.000
	Summer	07/19/2022-07/21/2022	434	18	0	0.000	0.000
SAJ-01	Spring	04/26/2021-04/28/2021	1,328	16	6	4.518	0.375
	Fall/winter	11/16/2021-11/18/2021	1,342	20	6	4.471	0.300
	Summer	06/07/2022-06/09/2022	750	20	2	2.667	0.100
	Fall/winter	10/19/2022-10/21/2022	1,251	20	1	0.799	0.050
SAJ-03	Spring	05/10/2021-05/11/2021*	696	8	4	5.747	0.500
	Summer	09/13/2022-09/15/2022	1,182	20	5	4.230	0.250
SAJ-04	Fall/winter	10/18/2021-10/20/2021	1,862	20	0	0.000	0.000
	Summer	08/10/2022-08/12/2022	1,828	20	1	0.547	0.050
SAJ-05	Spring	04/20/2021-04/22/2021	1,589	16	0	0.000	0.000
	Summer	06/22/2021-06/24/2021	1,246	20	2	1.605	0.100
	Fall/winter	10/20/2021-10/22/2021	1,514	20	4	2.642	0.200
	Spring	02/08/2022-02/10/2022	1,661	20	0	0.000	0.000
	Summer	08/08/2022-08/10/2022	1,259	20	0	0.000	0.000
	Fall/winter	10/25/2022-10/27/2022	1,568	20	4	2.551	0.200
SJB-03	Summer	06/09/2021-06/11/2021	1,564	20	0	0.000	0.000
	Summer	09/27/2021-09/29/2021	1,888	20	0	0.000	0.000
SJB-05	Spring	04/13/2021-04/14/2021*	2,636	8	0	0.000	0.000
	Summer	09/13/2022-09/15/2022	2,738	20	0	0.000	0.000
SUB-01	Summer	09/27/2022-09/29/2022	1,510	20	0	0.000	0.000
SUB-02	Summer	09/26/2022-09/28/2022	1,265	20	0	0.000	0.000

Table 6 Survey sites, season sampled, survey date(s), total reach length in river kilometers (r-km), effort in number of trap nights, total number of Alligator Snapping Turtles (AST; *Macrochelys temminckii*), density of AST as number of turtles per r-km, and catch per unit effort (CPUE, number of turtles per trap night) for each survey event conducted in the current study. Surveys were conducted from April 2021–November 2022. Asterisks (*) indicate events that were terminated early due to local conditions.

Site ID	Season	Survey dates	Reach length (r-km)	Effort	Total # AST	AST Density	AST CPUE
SUB-05	Summer	06/28/2022-06/30/2022	1,149	20	0	0.000	0.000
	Fall/winter	10/05/2022-10/07/2022	742	20	0	0.000	0.000
TRB-01	Spring	05/13/2021-05/15/2021	1,321	16	8	6.056	0.500
	Spring	05/09/2022-05/11/2022	1,301	20	3	2.306	0.150
	Fall/winter	11/02/2022-11/04/2022	984	20	0	0.000	0.000
TRB-02	Summer	08/10/2021-08/12/2021	1,126	20	0	0.000	0.000
TRB-04	Fall/winter	11/09/2021-11/11/2021	680	20	1	1.471	0.050
	Spring	03/09/2022-03/10/2022*	704	10	0	0.000	0.000
	Summer	07/25/2022-07/27/2022	819	20	1	1.221	0.050
	Fall/winter	11/15/2022-11/17/2022	1,253	20	0	0.000	0.000
TRB-05	Summer	08/23/2021-08/25/2021	1,960	20	3	1.531	0.150
	Fall/winter	01/25/2022-01/27/2022	2,368	20	0	0.000	0.000
	Summer	06/14/2022-06/16/2022	1,225	20	1	0.816	0.050
	Fall/winter	10/25/2022-10/27/2022	1,564	20	0	0.000	0.000
TRB-16	Summer	09/14/2021-09/16/2021	1,518	20	0	0.000	0.000
		Total (*n*)	111,380	1558	78	--	--
		Average ± 1 SE	1,341.9 ± 72.35	18.8 ± 0.33	0.9 ± 0.19	0.772 ± 0.1539	0.053 ± 0.0116

Population Genetics

We successfully sequenced 1.182 billion reads from 225 AST (median = 5.835 million reads; range = 1022 to 17.74 million). We removed 11 turtles that had fewer than 1 million reads successfully sequenced, and then three individuals that had fewer than 2,000 loci from *ipyrad*, leaving 215 turtles for our final dataset. The final dataset contained 571,259 unfiltered SNPs on 196,109 RAD loci. Subsequent analyses used a smaller subset of these SNPs as they required SNPs to be present in certain proportions of all individuals.

Principal Component Analysis (PCA)

The PCA containing all 215 individuals contained 45,440 SNPs and showed three major groupings (Figure 19). One group included individuals originating from the Red, Cypress, and Sulphur River drainages (herein referred to as R+C+S). Another included individuals originating from Sabine and Neches River drainages (herein referred to as Sa+N). The third included individuals originating from Trinity and San Jacinto River basins (herein referred to as SJ+N). While most individuals fit into these three groupings, some individuals did not fit the pattern. Three individuals from Turtle Bayou in the Trinity basin clustered with individuals from the Sa+N metapopulation while individuals from Buffalo Bayou in the San Jacinto basin near Houston showed affinities to the R+C+S metapopulation.

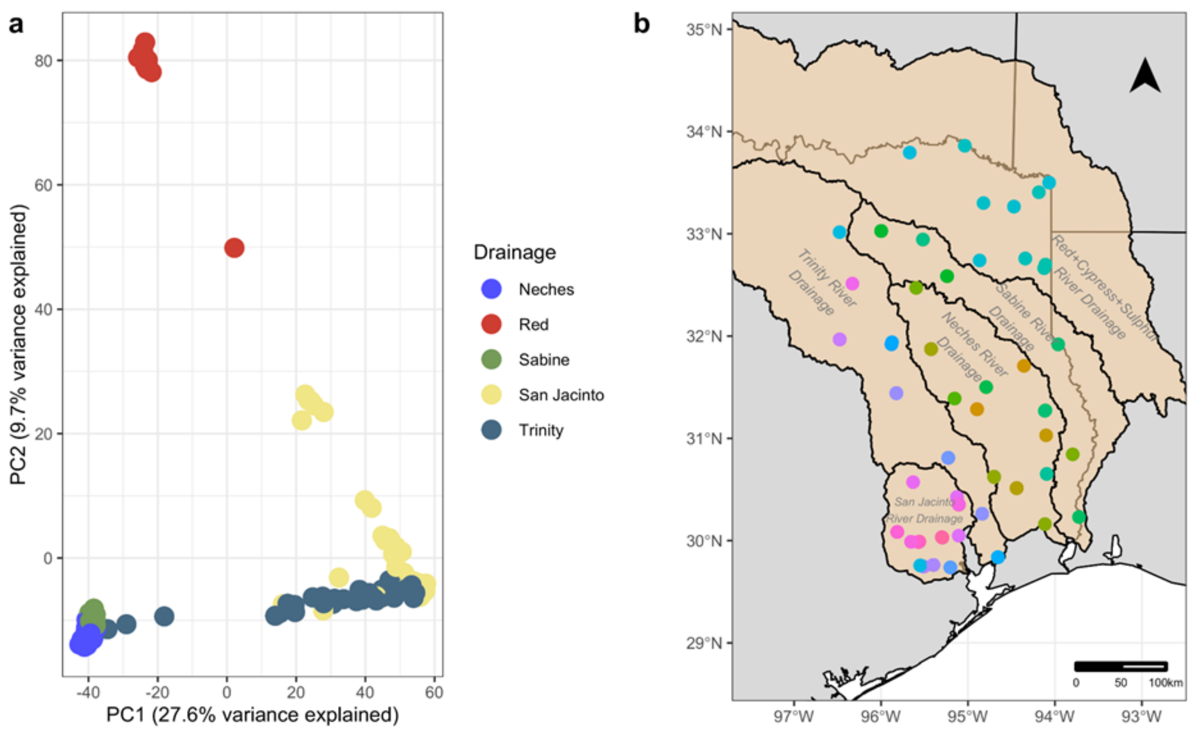


Figure 19 Principal Component Analysis (PCA) of 215 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in PC-space (a), and on a map (b). Overall, PCA is agnostic to population grouping. Individuals are colored by river drainage. Individuals cluster by river drainage, with drainages that share an outlet bay being more closely related to each other. The points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.

While we evaluated the same PCAs at a higher resolution (by basin of origin). Sample sizes were insufficient to extrapolate meaningful relationships. The San Jacinto PCA contained 60 individuals and 14,650 SNPs showing that individuals from Buffalo Bayou had the most variation, occupying the widest range on PC1. The Trinity PCA contained 40 individuals and

12,776 SNPs. Here, individuals from Turtle Bayou had the most variation, occupying the widest range on PC1. The Neches PCA included 77 individuals and 36,977 SNPs revealing no one population with the largest variation along PC1 or PC2. The Sabine PCA included 20 individuals and 29,226 SNPs with Cowleech Creek showing the greatest variation along PC1 and PC2. Finally, the R+C+S PCA included 18 individuals and 20,401 SNPs but also showed no single population with a large amount of variation along PC1 or PC2. Full results of the basin-level evaluation can be found in Appendix C, but for the purpose of this report, we present remaining population genetic results at the metapopulation-level.

Structure analysis

We used 30,064 SNPs in fastSTRUCTURE analyses and revealed that the three (meta)populations (K = 3) identified in PCA analyses best represented the dataset (Figure 20).

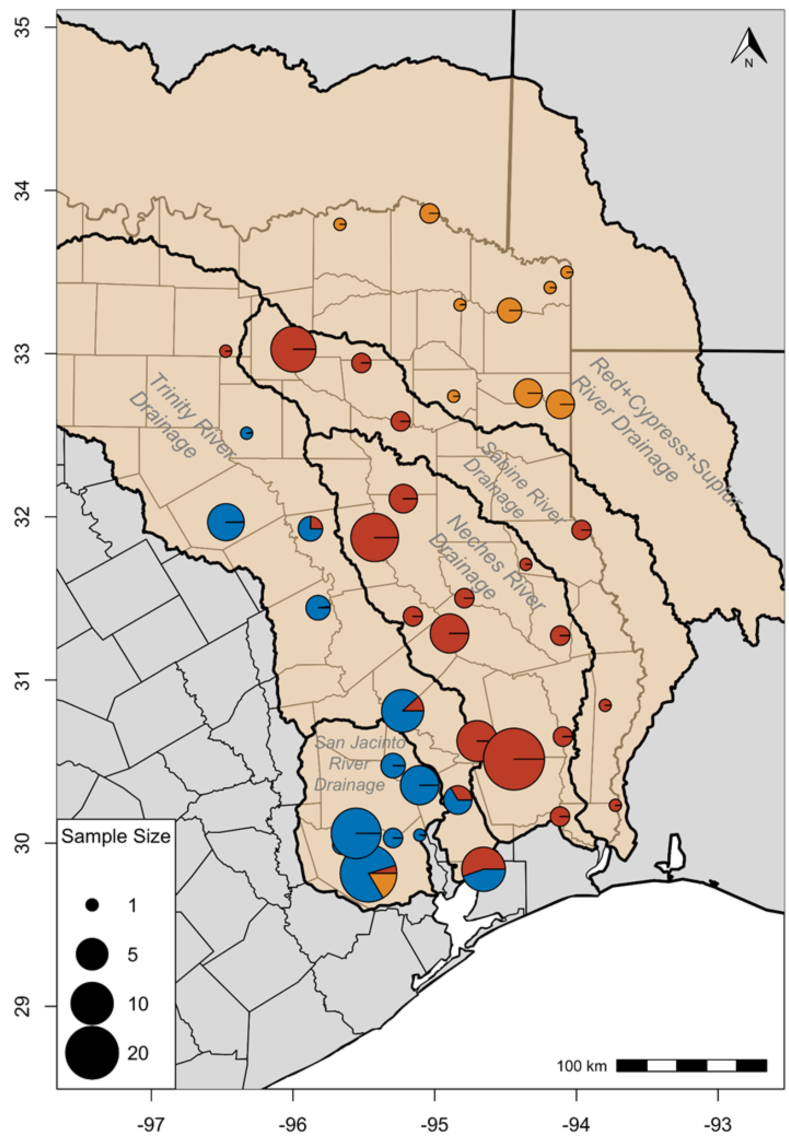


Figure 20 Plot of fastSTRUCTURE analyses for each locality in Texas. Pies represent mean admixture proportions for the most likely number of populations that describe the dataset (K = 3) at each sampling locality. The size of each pie chart is scaled by the sample size. Information about an individual's river drainage was not run in the fastSTRUCTURE analyses, but river drainages are shown here to highlight likely boundaries of the populations.

The R+C+S and Sa+N metapopulations showed little mixing with the other populations, but the SJ+T population did show some admixture, particularly in Buffalo Bayou near Houston, Turtle Bayou near the mouth of the Trinity River, and in the upper Trinity watershed.

Genetic diversity, population subdivision, and effective population size

We used the same dataset as the fastSTRUCTURE analyses for analyses of genetic diversity, population subdivision and effective population size (215 individuals, 30,064 SNPs). While all effective population sizes were low, the SJ+T metapopulation showed the lowest effective population size (N_e = 25.9 turtles) (Table 7). Individuals originating from the Trinity River basin also showed 10% fewer heterozygotes than expected under Hardy-Weinberg equilibrium, indicating that inbreeding may be occurring in this drainage (see Appendix C). Population subdivision, as measured by F_{ST}, was strong among populations identified by fastSTRUCTURE analyses and among river drainages (Table 7 and 8). Among populations identified by fastSTRUCTURE, F_{ST} values ranged from 0.311 to 0.453.

Table 7 Sample size (n), observed heterozygosity (H_O; Nei 1987), within-population gene diversity (sometimes referred to as expected heterozygosity, H_S; Nei 1987), within-population subdivision (F_{IS}; Nei 1987), and effective population size (N_e; Waples and Feutry 2021) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in Texas. Statistics are calculated for the three metapopulations determined by fastSTRUCTURE analysis (Red+Cypress+Sulphur, Sabine+Neches, and Trinity+San Jacinto).

Population	n	H_O	H_S	F_{IS}	N_e
Sabine+Neches (Sa+N)	97	0.0873	0.0904	0.0339	174.5 (117.6 - 311.2)
San Jacinto+Trinity (SJ+T)	100	0.0777	0.0864	0.1002	25.9 (25.9 - 25.9)
Red+Cypress+Sulphur (R+C+S)	16	0.0696	0.0698	0.0038	444.4 (411.7 - 482.7)

Table 8 Population subdivision (F_{ST}) for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) populations in Texas as identified by fastSTRUCTURE analyses. See Table 7 for sample sizes.

Population F_{ST} values	Red+Cypress+Sulphur (R+C+S)	Sabine+Neches (Sa+N)	San Jacinto+Trinity (SJ+T)
Red+Cypress+Sulphur (R+C+S)	NA	0.3885	0.4528
Sabine+Neches (Sa+N)	0.3885	NA	0.3110
San Jacinto+Trinity (SJ+T)	0.4528	0.3110	NA

Effect of dams on genetic connectivity

We were able to run permutation tests at Lake Livingston (Palmetto Creek [above]: n = 10; Little Bayou [below]: n = 4), Richland-Chambers Reservoir (Pin Oak Creek [above]: n = 7; Keechi Creek [below]: n = 3), Lake Palestine (Lake Palestine [above]: n = 3; Dead Water Lake [below]: n = 14), and Lake Tawakoni (n = 12 from Cowleech Creek above, n = 2 from Little Sandy Hunting and Fishing Club below), though we did not find any significant impact of dams on genetic connectivity. Overall population subdivision across dams was low, similar to the population subdivision across individual drainages (Appendix C). Localities across the Lake Livingston Dam had an F_{ST} of 0.002 (p = 0.36), while Richland-Chambers, Lake Palestine, and Lake Tawakoni had F_{ST}'s of 0.027 (p = 0.10), 0.023 (p = 0.06), and 0 (p = 0.95), respectively.

Sex-linked marker discovery

We ran sexy_markers on a dataset of 77 individuals from the Neches River drainage (n = 16 males, n = 17 females, n = 44 unknown sex), with a total of 571,259 SNPs. We did not successfully recover any markers that segregated perfectly among the sexes.

Physiological Results

Morphometric results

In addition to the 69 unique individuals captured in this study, we compiled an additional 131 previously unpublished measurements from collaborators and key contributors (Appendix D). While we received measurements for 30 AST hatchlings (29 of which were from a single nest), we excluded the hatchling age class from morphometric analyses. We did not detect significant differences between metapopulation for males or females, but data for unknown sex resulted in multiple significant differences between metapopulations. For consistency between the way data are reported in the current study to those from previous studies, we pooled morphometric data for individuals of unknown sex (Table 9), but recommend that this be evaluated further in future assessments. For all measurements reported, median values for males were larger than females and females larger than individuals of unknown sex. To further test the applicability of our proposed age-size class structure, we evaluated mid-SCL in relation to pre-C between females with sex confirmed by observation of reproductive structures using ultrasonography and individuals identified as males based on observed diagnostic characteristics in the field (Figure 21). Overall, mid-SCL and pre-C were less correlated in females than males ($R^2 = 0.1143$ and 0.7249, respectively), but the intercept of regression lines for each group occurred around 347 mm mid-SCL and 80 mm pre-C.

Observed traits, abnormalities, injuries, and epibionts

Shell algae (e.g. epiphytic growth) was the most commonly observed external characteristic for all sexes (Table 10). Leeches (Figure 22) were the only external parasite observed throughout this study and were documented on 50% of all ASTs captured. Shell damage was common across all sexes, but males exhibited major damage to appendages more than females and juveniles. Other external characteristics observed included damage to the head ($n = 4$ males), missing "eyelashes" ($n = 2$ males), scarring from an old bite wound ($n = 1$ male), a growth on the dorsal surface of the neck ($n = 1$ male), sloughing or peeling skin ($n = 8$ males, $n = 2$ females), and damage to the front right foot ($n = 1$ female) (Table 10).

Metal detection preliminary results

We scanned 55 AST for presence of foreign metallic objects and detected metal in six (10.91%) individuals. In one individual, presence was detected in the abdomen, while presence was detected in the neck of three other individuals (Table 11). We were unable to visualize metallic objects using ultrasonography and, ultimately, the identity of foreign metallic objects could not be confirmed. In two individuals, a metal fishing hook was visually observed in the back of the mouth which produced a positive detection in the external portion of the same area.

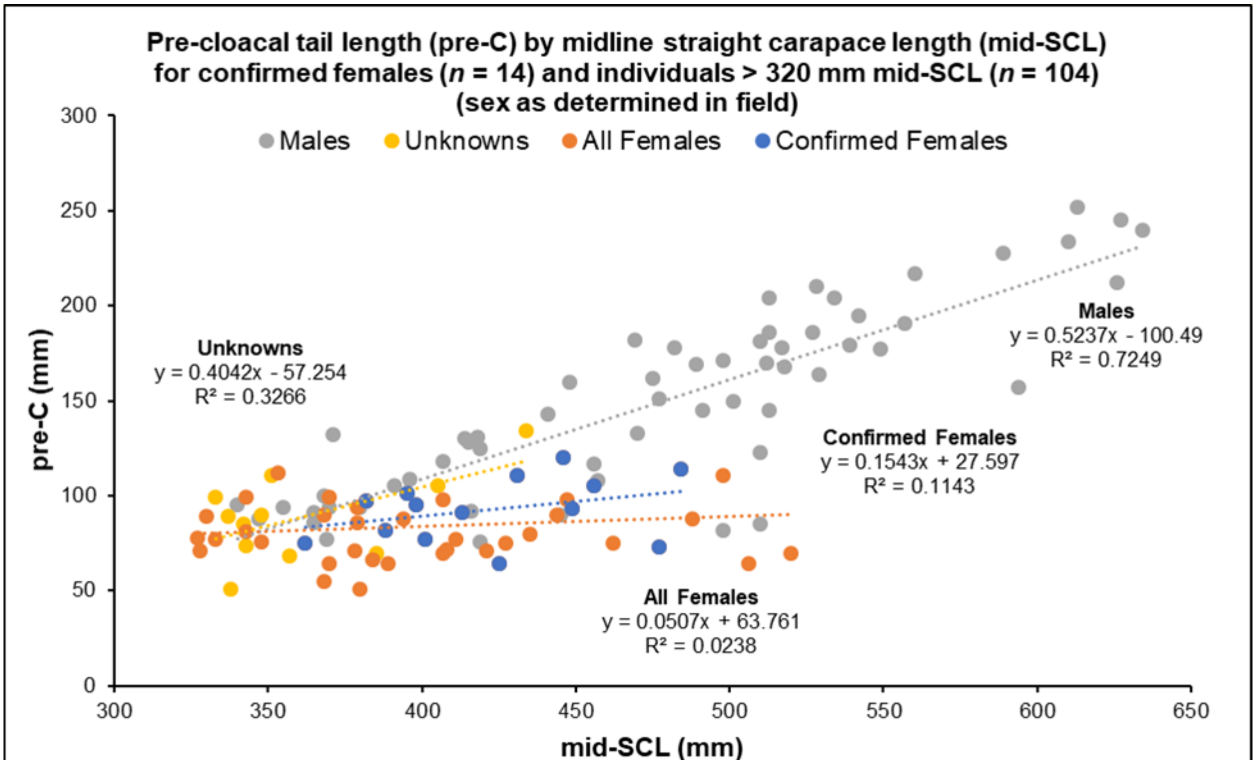
Sonographic results

We assessed 26 females using ultrasonography (Figure 23). Among these, 12 were quiescent across varying dates (Figure 24). The smallest maximum follicle diameter (1.32 cm) was observed on 5 August 2021 and the largest on 20 March 2022 (2.80 cm), along with some follicles that appeared to in the initial stages of shell development. One large follicle (2.31 cm diameter) was observed in the left ovary along with smaller follicles on 10 June 2021, but the right ovary was quiescent. Follicular development was positively correlated with Julian date from August to November ($R^2 = 0.7434$, $p < 0.001$). Of females in which reproductive structures were observed, mid-SCL ranged from 348–484 mm mid-SCL (average = 421.0 mm) while individuals in which structures were not observed ranged from 205–498 mm mid-SCL (average = 340.7 mm). Through the duration of the study, we failed to detect any fully shelled eggs.

Table 9 Summary of morphological measurements for Alligator Snapping Turtles (AST; *Macrochelys temminckii*) compiled for the current report. Test and significance (p) values presented for each morphometric measurement. Significant groupings based on pairwise comparisons.

	Females	Males	Unknown	Test Value	p-value	Groupings
Current Study						
Count of unique individuals (n)	52	62	56			
Carapace length (mid) mm)	402.7 ± 7.49 (294-520)	470.0 ± 10.73 (308-634)	243.8 ± 11.41 (91-434)	H = 104.754	< 0.001	M > F > U
Carapace length (max) mm)	425.9 ± 7.72 (311-541)	507.9 ± 12.16 (332-697)	260.8 ± 12.86 (101-475)	H = 100.696	< 0.001	M > F > U
Carapace width (max) mm)	334.7 ± 5.79 (242-430)	397.2 ± 8.83 (262-544)	212.6 ± 9.77 (85-376)	H = 102.945	< 0.001	M > F > U
Head width (mm)	125.2 ± 2.52 (91-165)	149.5 ± 3.85 (95-214)	77.8 ± 3.65 (29-129)	H = 98.844	< 0.001	M > F > U
Shell depth (max) mm)	157.8 ± 4.58 (115-332)	182.3 ± 7.18 (117-396)	98.5 ± 5.78 (41-264)	H = 81.168	< 0.001	M > F > U
Plastron length (mid) mm)	306.5 ± 5.88 (210-388)	347.2 ± 7.28 (238-464)	182.7 ± 8.79 (71-321)	H = 101.668	< 0.001	M > F > U
Plastron width (outer) mm)	278.0 ± 10.53 (35-355)	329.6 ± 9.51 (140-444)	181.4 ± 8.66 (69-313)	H = 72.495	< 0.001	M > F > U
Pre-cloaca (mm)	84.0 ± 2.27 (51-120)	146.0 ± 6.38 (63-252)	59.0 ± 3.52 (19-134)	H = 94.12	< 0.001	M > F > U
Mass (kg)	15.36 ± 0.875 (4.9-32.2)	25.43 ± 1.706 (7.2-56.8)	4.76 ± 0.596 (0.2-20.1)	H = 98.368	< 0.001	M > F > U
Rudolph et al (2002) - Table 6						
Number reported (n)	21	19	8	--	--	--
Mean carapace length (mm)[a]	412.0 ± 7.24 (351-461)	466.0 ± 21.13 (304-583)	251.0 ± 16.51 (155-290)	NR	NR	M > F > U
Mass (kg)	16.80 ± 0.869 (10.2-23.8)	25.30 ± 3.093 (6.8-46.5)	4.10 ± 0.647 (0.8-6.0)	NR	NR	M > F > U
Riedle (2014) - Table 3.3						
Number reported (n)	1	0	12	--	--	--
Mid-line carapace length (mm)	319	None collected	206.7 ± NR (44-287)	NR	NR	NR
Munscher et al. (2023) – Table 3						
Number reported (n)	40	50	NR	--	--	--
Carapace length mid (mm)	421.0 (287-517)	521.3 (289-647)	NR	t = 6.2	< 0.001	M > F
Carapace length (max) (mm)	444.8 (300-558)	559.8 (342-683)	NR	t = 7.0	< 0.001	M > F
Carapace width (mm)	353.7 (238-443)	433.8 (245-526)	NR	t = 6.4	< 0.001	M > F
Plastron length max (mm)	324.1 (200-414)	381.5 (208-468)	NR	t = 4.9	< 0.001	M > F
Shell height (mm)	157.4 (106-194)	190.7 (112-224)	NR	t = 6.4	< 0.001	M > F
Head width (mm)	129.0 (88-167)	164.4 (100-207)	NR	t = 6.7	< 0.001	M > F
Pre-cloaca length (mm)	90.8 (55-127)	190.2 (86-263)	NR	t = 9.3	< 0.001	M > F
Mass (g)	19,237.0 (5,000-33,700)	34,952.6 (8,600-59,800)	NR	t = 6.6	< 0.001	M > F
Nelson (1999) - Table 1[b]						
Number reported (n)	9	17	9	--	--	--
Mass (kg)	17.30 ± 2.200 (6.5-25.5)	18.60 ± 2.401 (7.3-36.7)	3.90 ± 0.733 (0.7-9.5)	$t_{df = 25} = 0.417$	0.34	M = F
Midline carapace length (mm)	411.0 ± 18.33 (316-469)	418.0 ± 17.22 (312-550)	244.0 ± 16.00 (145-335)	$t_{df = 25} = 0.326$	0.37	M = F
Shell width (mm)	346.0 ± 14.67 (283-411)	361.0 ± 14.07 (279-473)	204.0 ± 12.33 (127-238)	NR	NR	NR
Shell depth (mm)	157.0 ± 9.67 (114-204)	162.0 ± 6.55 (121-202)	91.0 ± 6.00 (53-107)	NR	NR	NR
Skull width (mm)	126.0 ± 7.00 (101-162)	133.0 ± 5.58 (102-187)	76.0 ± 4.00 (49-86)	NR	NR	NR

[a]Converted from centimeters (cm)
[b]Also presented as Table 6 in Rudolph et al. (2002)



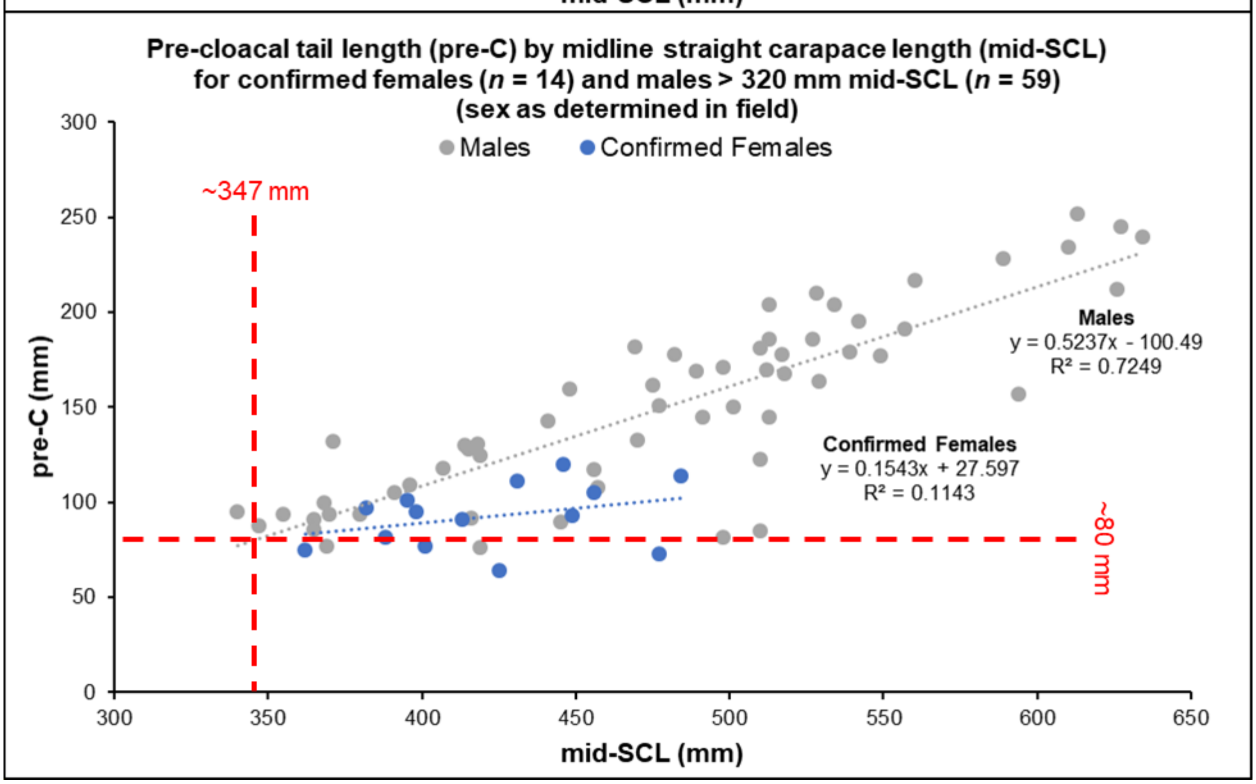


Figure 21 Midline straight carapace length (mid-SCL, in millimeters) versus pre-cloacal tail length (pre-C, in millimeters) for females where sex was confirmed by observation of reproductive structures using ultrasonography (blue dots and dashed trendline) and individuals within the proposed "adult" age-class based on mid-SCL (> 320 mm). Top: all individuals > 320 mm mid-SCL (*n* = 118) with associated linear regression equations and trendlines. Bottom: confirmed females and males (as identified in the field) with intercept of linear regression denoted by dashed red-line (near 347 mm mid-SCL and 80 mm pre-C).

Table 10 External injuries and abnormalities observed during the current study of Alligator Snapping Turtle (AST; *Macrochelys temminckii*) in Texas. Listed from highest to lowest occurrence for all turtles captured (*N* = 80).

Characteristic observed	Females Number (*n*)	Females Percent	Males Number (*n*)	Males Percent	Juveniles Number (*n*)	Juveniles Percent	Total (*N*)	Percent of Total
Shell algae	16	25.4	23	36.5	24	38.1	63	78.8
External parasites	5	12.5	18	45.0	17	42.5	40	50.0
Damage to shell	7	30.4	9	39.1	7	30.4	23	28.8
Other	4	19.0	15	71.4	2	9.5	21	26.3
Scute sloughing	4	22.2	4	22.2	10	55.6	18	22.5
Tail damage	3	30.0	7	70.0	0	0.0	10	12.5
Lesion	1	14.3	3	42.9	3	42.9	7	8.8
Missing digit(s)	1	20.0	4	80.0	0	0.0	5	6.3
Supramarginals absent	1	100.0	0	0.0	0	0.0	1	1.3
Missing limb(s)	0	0.0	1	100.0	0	0.0	1	1.3

Figure 22 Adult female Alligator Snapping Turtle (AST; *Macrochelys temminckii*) with heavy external parasitic leech load on posterior dorsal surface of neck (left). Adult male AST with missing posterior right limb.

Table 11 Observations of metallic foreign bodies detected with a metal detector during Alligator Snapping Turtle (AST; Macrochelys temminckii) surveys in east Texas. Includes sex, straight carapace length (SCL; in mm), county, date of observation, and location within the body. Yes in "Visual" column indicates that metallic object was visually observed in conjunction with a positive detection by the metal detector; type of foreign metallic body indicated in "Type" column.

Sex	SCL (mm)	County	Date	Location	Visual	Type
M	482	Hardin	05/14/2022	Abdomen	No	Undetermined
M	628	Chambers	07/26/2022	Neck	No	Undetermined
F	488	Harris	09/29/2022	Neck	No	Undetermined
F	449	Harris	10/27/2022	Neck	No	Undetermined
F	379	Harris	01/17/2022	Mouth	Yes	Fishing hook
M	604	Harris	02/22/2023	Mouth	Yes	Fishing hook

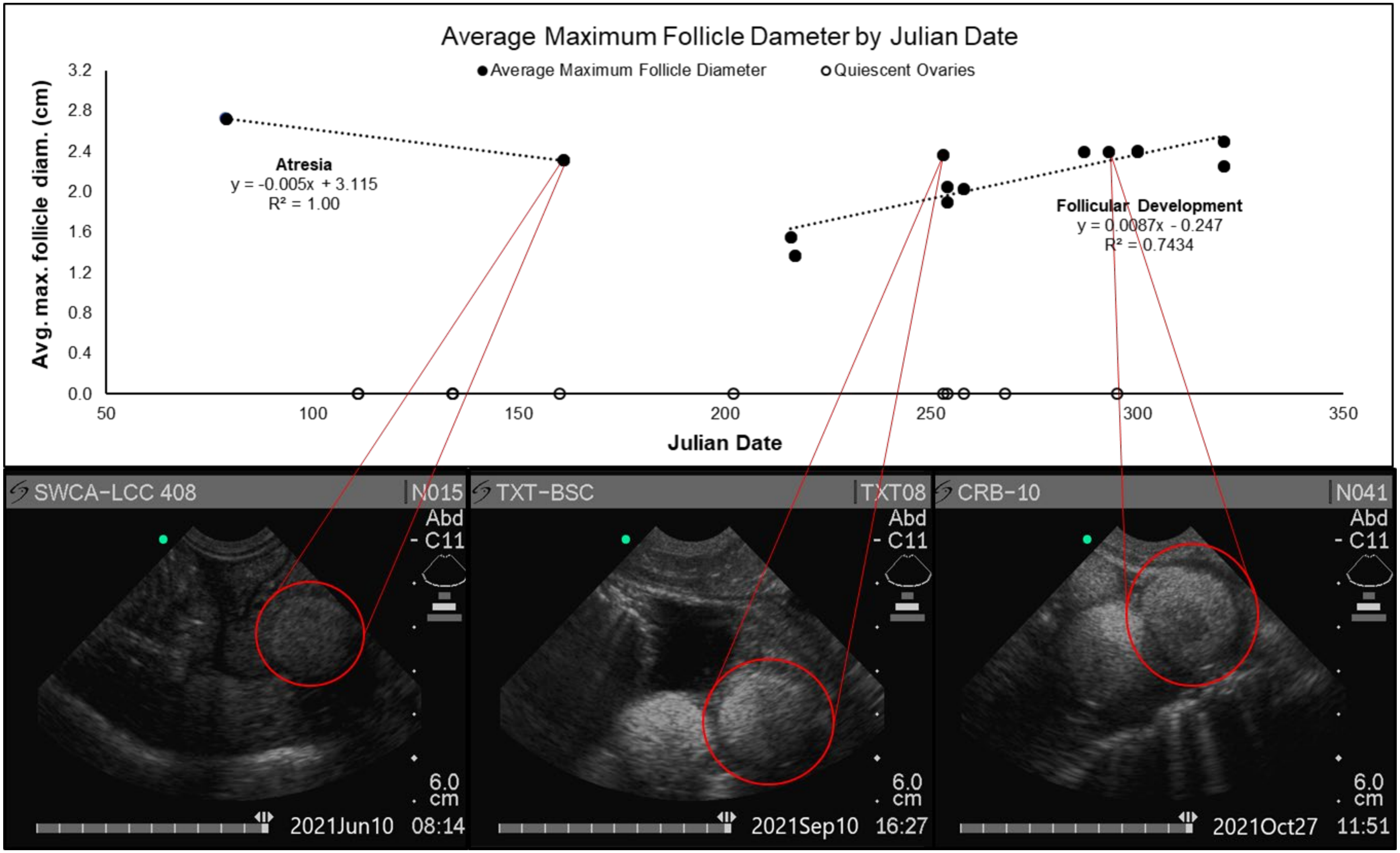


Figure 23 Average maximum follicle diameter as detected by ultrasound from female Alligator Snapping Turtles (AST; *Macrochelys temminckii*) captured during the current study ($n = 26$). Corresponding follicles circled in ultrasound images below graph. Follicular development was correlated with Julian date from August through November ($R^2 = 0.7434$, $p < 0.001$). A large follicle was observed in the left ovary of a female on 10 June 2021 while the right ovary was quiescent on the same date. A second individual captured 20 March 2022 was observed with some follicles beginning to shell. Across all females assessed, 12 were quiescent at varying times in the survey period.

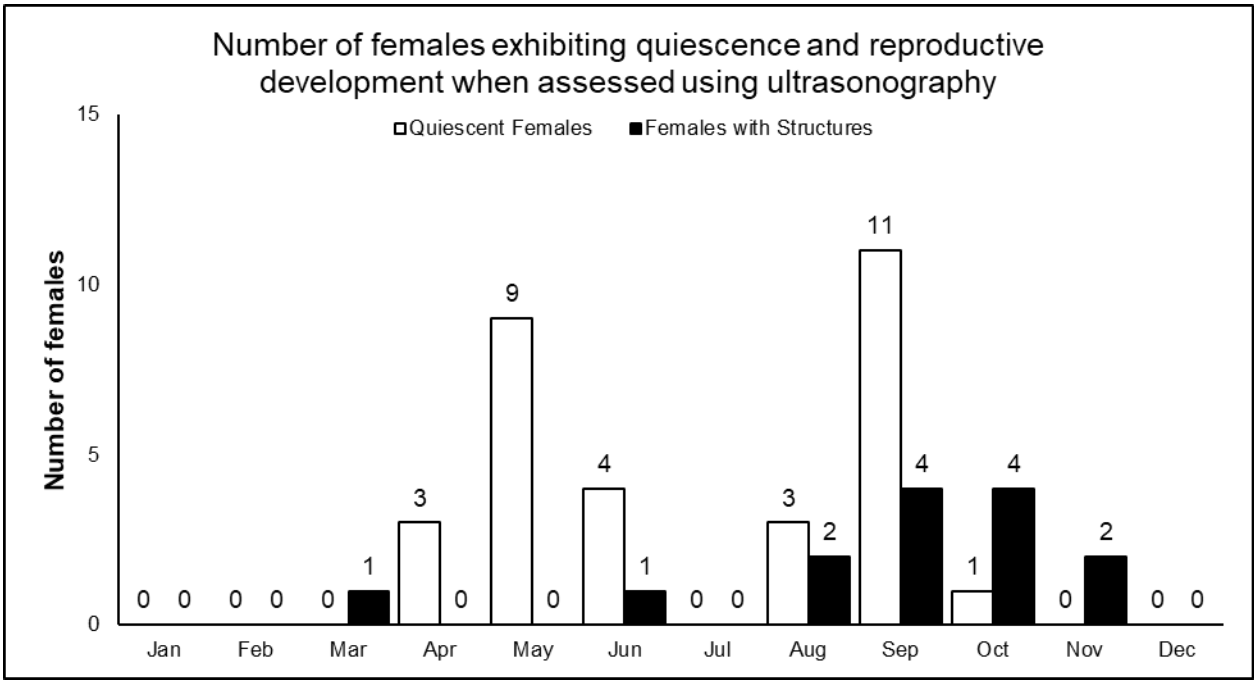


Figure 24 Number of female Alligator Snapping Turtles (AST; *Macrochelys temminckii*) exhibiting quiescence (lack of reproductive structures) and development (e.g., follicles or eggs) when assessed during ultrasonography in the current study. Numbers above bars represent sample size.

Habitat Associations

Environmental and habitat variables

Environmental and habitat variables were compared to determine any significant correlations with AST detections (Table 12). Dissolved oxygen, thalweg depth, percent bareground cover, water temperature, and in-water cover associated with deadfall, logs, and woody debris were significantly correlated with AST detection. Dissolved oxygen (DO, mg/L) was increased at sites where ASTs were detected ($p = 0.041$) (Figure 25) with the probability of detection highest when DO was greater than 10 mg/L. Thalweg depth was also increased at sites where ASTs were detected ($p = 0.0482$) (Figure 26). The probability of detection nearly doubled at sites where thalweg depth was > 2-meters ($p = 0.089$). Exposed ground, observed as a percentage of two-dimensional area coverage around the trap, was also positively correlated with AST detection ($p = 0.049$; Figure 27), with probability appearing to plateau around 12% Water temperature was increased at sites where ASTs were not detected ($p = 0.010$; Figure 28a), with average temperature at sites without detection higher (26.09°C) than sites without detection (23.31°C). In-Water Cover matching the "other" criteria was increased at sites with AST detections ($p = 0.002$, Figure 28b), with an average coverage of 0.9% at sites with no detections and 2.1% at sites with detections.

Categorical data were also evaluated at the site level, including primary substrate, bank slope, waterbody type, and surrounding area type (Table 13). Sites with fines as the primary substrate type had the highest proportion of AST detections (0.875). Sites with steep or gradual bank slopes had the highest proportion of AST detections (0.444). Overall, riverine waterbody types surrounded by forested habitat comprised the largest proportion of sites where AST were detected.

Table 12 Summary of water quality and small-scale habitat variables for all sites sampled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the current study. Summary results presented for sites where AST were detected versus those where AST were not detected. Values are presented as average ± 1 standard error (SE) with range (minimum to maximum) in parentheses. Differences based on occupancy between variables were tested using one-way ANOVA (F-score) or Kruskal-Wallis one-way ANOVA on Ranks (H-score) with α = 0.05 and significant *p*-values italicized.

Variable	AST not detected	AST detected	Test statistic	*p*-value
Water Temperature (°C)	26.09 ± 0.882 (21.1-36.2)	23.31 ± 0.603 (17.7-27.1)	H = 6.696	*0.010*
Specific Conductivity (µS)	1445.68 ± 696.465 (101.4-8,959.4)	320.01 ± 57.799 (37.1-793.0)	H = 1.905	0.168
Dissolved Oxygen (mg/L)	5.361 ± 0.5154 (0.87-9.48)	6.584 ± 0.2895 (4.23-8.85)	F = 5.833	*0.041*
pH	7.50 ± 0.137 (6.2-8.4)	7.29 ± 0.114 (6.4-8.1)	H = 1.790	0.181
In water cover: SAV (%)	6.0 ± 3.09 (0-47)	2.4 ± 1.42 (0-25)	H = 0.097	0.755
In water cover: FAV (%)	2.1 ± 0.69 (0-11)	0.9 ± 0.36 (0-5)	H = 2.623	0.105
In water cover: Other (%)	6.4 ± 1.16 (2-23)	8.7 ± 0.7 (4-16)	H = 9.858	*0.002*
Thalweg depth (m)	1.4 ± 0.16 (1-3)	2.1 ± 0.29 (1-5)	H = 3.902	*0.048*
Secchi (m)	0.375 ± 0.0585 (0.111-0.938)	0.396 ± 0.0376 (0.127-0.720)	H = 0.744	0.388
Surrounding Depth (m)	0.81 ± 0.051 (0.6-1.4)	1.05 ± 0.078 (0.7-1.7)	H = 5.505	*0.019*
Surrounding area: tree (%)	34.9 ± 4.25 (0-58)	44.6 ± 3.93 (10-68)	F = 2.686	0.103
Surrounding area: shrubs (%)	1.5 ± 0.47 (0-5)	1.6 ± 0.27 (0-4)	H = 1.145	0.285
Surrounding area: grasses (%)	4.6 ± 1.27 (0-20)	3.2 ± 0.80 (1-12)	H = 0.344	0.557
Surrounding area: bareground (%)	2.5 ± 0.71 (0-10)	4.1 ± 0.67 (0-12)	H = 3.869	*0.049*
Surrounding area: open water (%)	51.4 ± 3.57 (32-90)	42.7 ± 3.01 (23-63)	F = 2.201	0.069
Total number of sites (n)	16	18	-	-

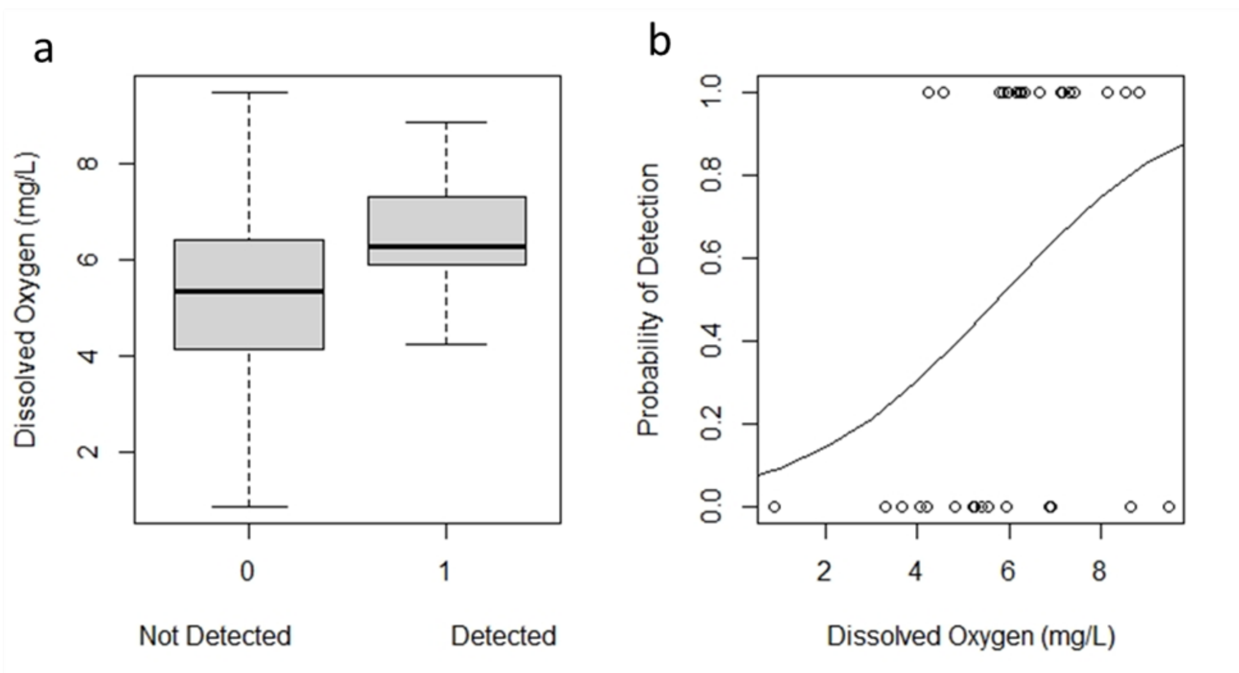


Figure 25 a) Boxplot of dissolved oxygen (DO, mg/L) where Alligator Snapping Turtles (AST; *Macrochelys temminckii*) were detected (1) versus not detected (0). Dissolved Oxygen was significantly higher at sites where AST were detected ($p = 0.041$). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of AST by dissolved oxygen with detection probability curve ($p = 0.057$).

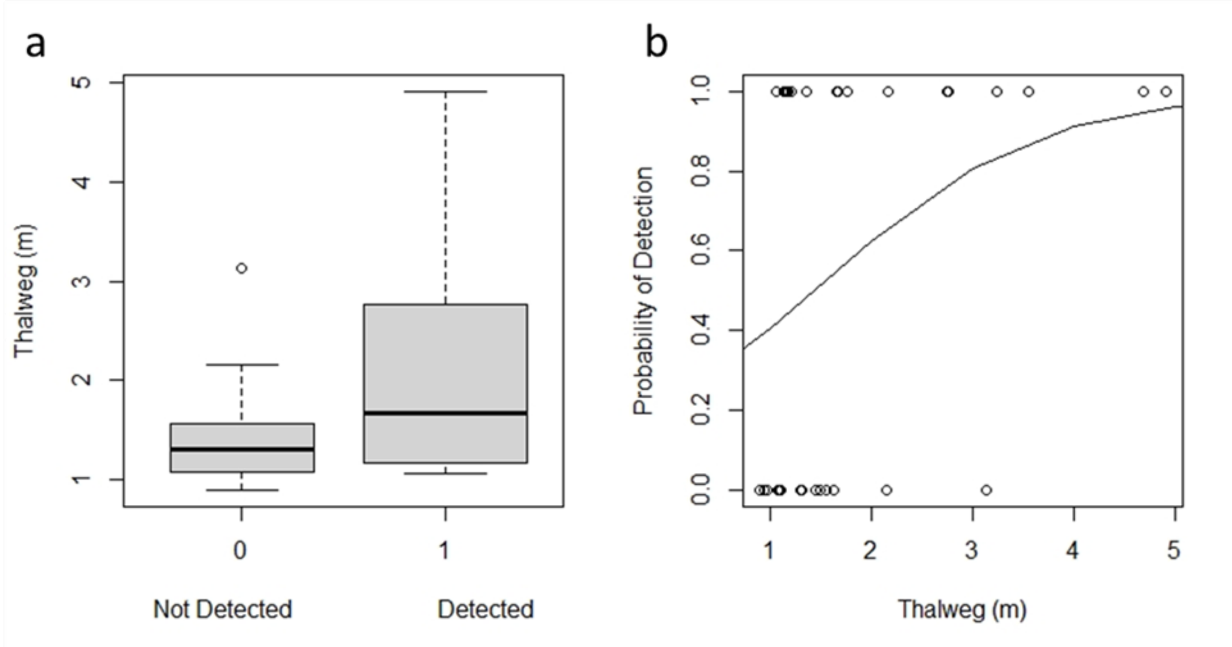


Figure 26 a) Boxplot of thalweg depth (m) where Alligator Snapping Turtles (AST; *Macrochelys temminckii*) were detected (1) versus not detected (0). Thalweg depth was significantly higher at sites where AST were detected (p = 0.048). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of AST by thalweg depth with detection probability curve (p = 0.089).

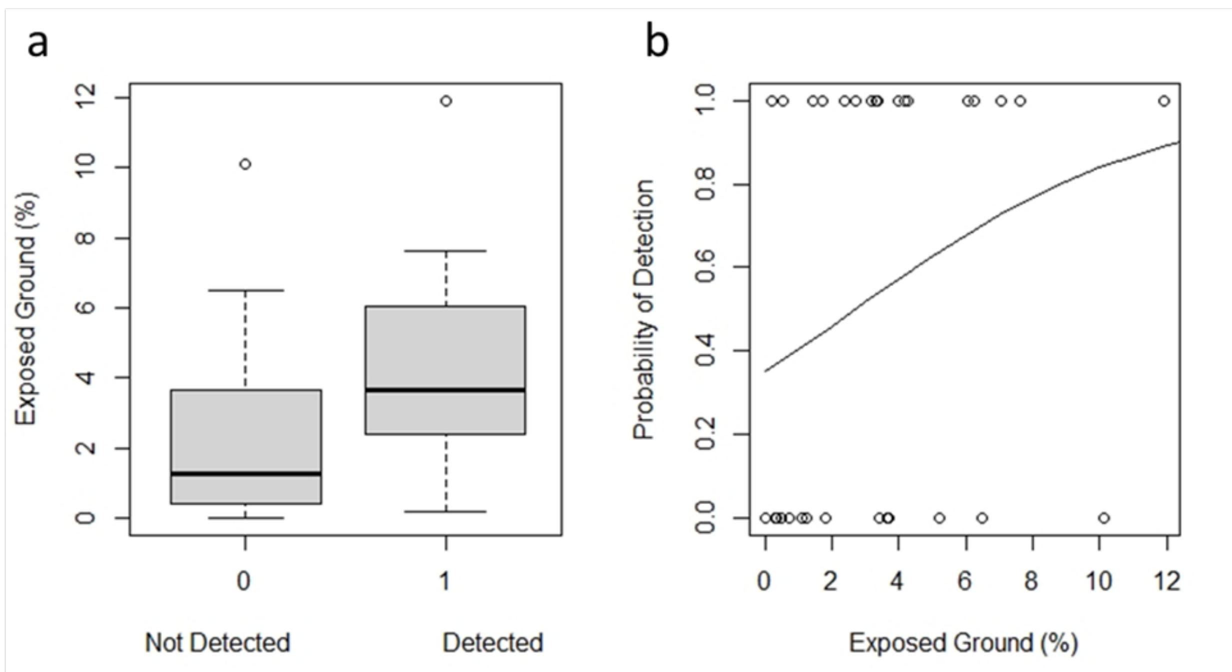


Figure 27 a) Boxplot of bareground cover (%) where Alligator Snapping Turtles (AST; *Macrochelys temminckii*) were detected (1) versus not detected (0). Exposed ground was significantly higher at sites where AST were detected (p = 0.049). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of detection of AST by exposed ground with detection probability curve (p = 0.113).

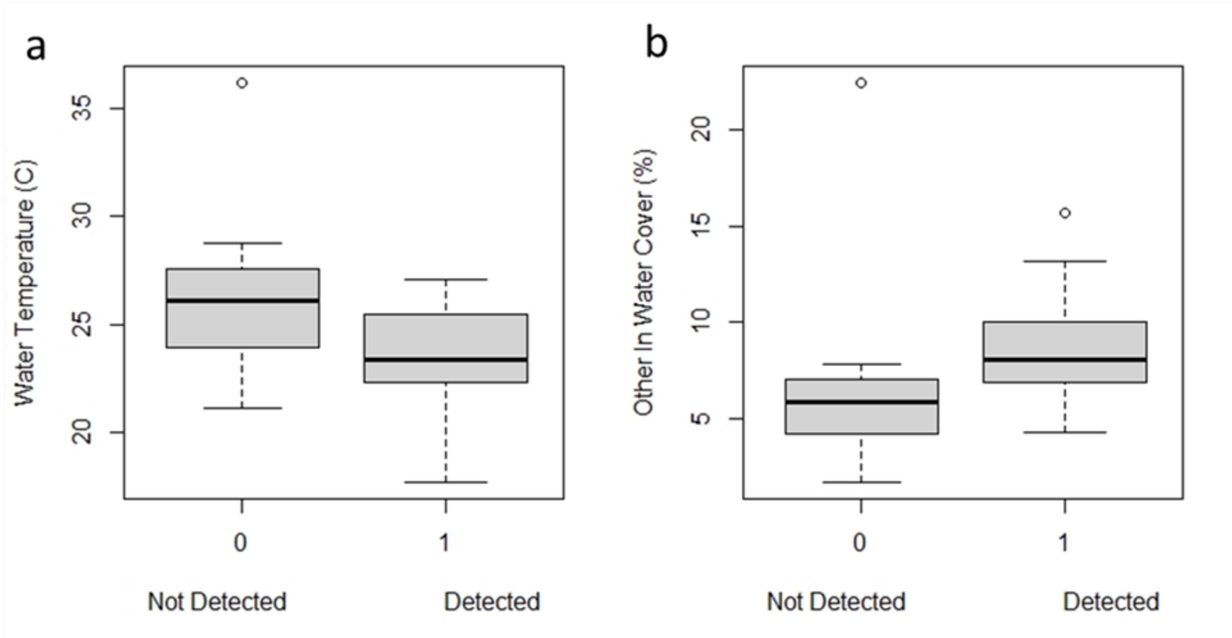


Figure 28 Boxplots of a) water temperature (°C) and b) in water cover matching the "other" category description (i.e. deadfall, woody debris, roots, etc.) where Alligator Snapping Turtles (AST; *Macrochelys temminckii*) were detected (1) versus not detected (0). Water temperature was significantly lower at sites where AST were detected ($p = 0.010$) while "other" in water cover was significantly higher at sites where AST were detected ($p = 0.002$).

Table 13 Proportion of events with and without Alligator Snapping Turtles (AST; *Macrochelys temminckii*) detections for categorical habitat variables. Categories variable with the highest proportion of confirmed AST detections for each are italicized.

Parameter	Category	Detections	No Detection
Substrate	*Fines*	*0.875*	*0.611*
Substrate	Detritus	0.063	0.000
Substrate	Sand	0.063	0.333
Substrate	Gravel	0.000	0.056
Bank Slope	*Steep*	*0.444*	*0.188*
Bank Slope	*Gradual*	*0.444*	*0.313*
Bank Slope	Near Vertical	0.056	0.000
Bank Slope	Undercut	0.056	0.188
Bank Slope	Vertical	0.000	0.125
Bank Slope	Flat	0.000	0.188
Waterbody Type	*Riverine*	*0.944*	*0.813*
Waterbody Type	Lake	0.056	0.188
Surrounding Area	*Forest*	*0.778*	*0.750*
Surrounding Area	Park	0.111	0.125
Surrounding Area	Rural/Pasture	0.111	0.125

Anthropogenic influences in the sample areas

Active or derelict fishing gear were observed at 29 of 34 sites (85.3%) during the current study while access points were present at 10 of 24 sites (41.7%) (Table 14). At sites occupied by ASTs, active or derelict fishing gear were observed at 89.5% (17 sites) and access points were observed at 37.5% (6 sites). Jenks Natural Breaks Optimization identified four ranges of gear counts correlated to low (one to nine cumulative fishing types observed), moderate (10-23 cumulative fishing types observed), and high (24+ cumulative fishing types observed) (goodness variance of fit = 85.9%). No significant difference in CPUE was observed between gear presence categories ($p = 0.468$) or between presence and absence of fishing gear ($p = 0.134$) (Figure 29). We failed to detect a significant effect on CPUE between sites with presence or absence of access points ($p = 0.381$).

Table 14 Surveyed sites with average count of observed fishing gear and human access points across all sampling events. Gear abundance categories were determined using Jenks Natural Breaks Optimization on the average number of gear types observed across visits. Average gear count includes range across multiple visits when applicable (NA = not applicable).

Site ID	Average gear count	Number of events	Gear abundance category	Average number of access points
BRB-02	5.0 (4-6)	2	Low	2
BRB-06	0.0 (0-0)	2	None	0
BRB-10	14.5 (10-19)	2	Moderate	0
CRB-04	1 (NA)	1	Low	0
CRB-09	3 (NA)	1	Low	0
CRB-10	21.8 (6-42)	4	Moderate	10
NRB-04	14.3 (9-23)	3	Moderate	0
NRB-07	10.3 (3-15)	3	Moderate	0
NRB-08	0.8 (0-2)	5	Low	0
NRB-09	4.7 (0-12)	3	Low	0
NTB-03	4.5 (2-7)	2	Low	0
NTB-06	9.0 (9-9)	2	Low	2
NTB-11	0.0 (0-0)	2	None	0
RRB-01	5 (NA)	1	Low	0
RRB-05	0.0 (0-0)	3	None	3
RRB-06	10 (NA)	1	Moderate	3
SAB-02	3 (NA)	1	Low	0
SAB-04	0.5 (0-2)	4	Low	0
SAB-05	11.0 (4-21)	3	Moderate	0
SAB-06	7.0 (0-11)	3	Low	0
SAJ-01	2.3 (0-6)	4	Low	0
SAJ-03	2.0 (0-4)	2	Low	1
SAJ-04	10.0 (0-20)	2	Moderate	0
SAJ-05	13.5 (1-30)	6	Moderate	12
SJB-03	6.0 (3-9)	2	Low	0
SJB-05	1.0 (0-2)	2	Low	2
SUB-01	4 (NA)	1	Low	0
SUB-02	47 (NA)	1	High	1
SUB-05	8.5 (7-10)	2	Low	0
TRB-01	0.0 (0-0)	3	None	0
TRB-02	0 (NA)	1	None	0
TRB-04	12 (3-34)	4	Moderate	11
TRB-05	0.8 (0-1)	4	Low	0
TRB-16	4 (NA)	1	Low	0

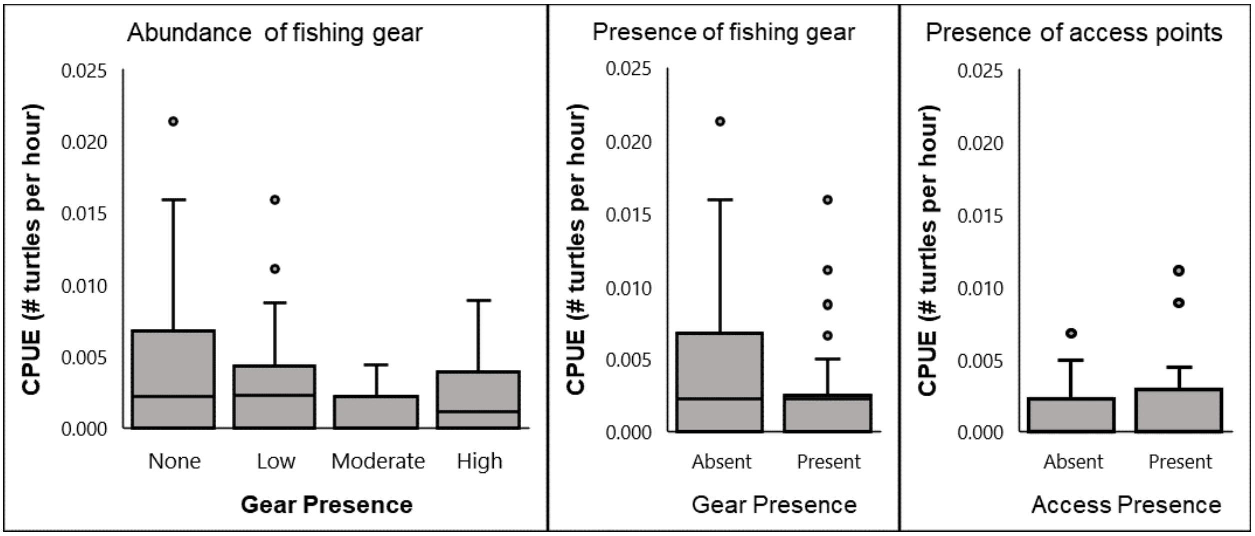


Figure 29 Catch per unit effort (CPUE; number of turtles per trap hour) for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in east Texas as a function of observed anthropogenic influences. *Left*: Calculated CPUE compared to gear presence by categories determined using Jenks Natural Breaks Optimization. *Middle*: Calculated CPUE compared to presence versus absence of fishing gear. *Righ*t: Calculated CPUE compared to presence of human access points (docks and ramps).

Associated Assemblages for Alligator Snapping Turtle Occupied Locations

Community data of aquatic vertebrates were analyzed for similarities and dissimilarities by site using PRIMER (version 7.0.21, PRIMER-e). Data were transformed by log(x+1) and an ANOSIM was conducted on the resemblance matrix. We detected significant differences (p = 0.001) between communities in which the AST presence was detected and not detected (Figure 30). Pearson correlations were used to evaluate the species which contributed the most to variances on the two-dimensional NMDS plot ordinations, resulting in AST as the second highest correlation to MDS2 ordination (+0.390). All MDS2 Pearson Correlation Values greater than ±0.25 are included in Figure 30. A full list of by-catch can be found in Appendix E.

A cluster analysis was also conducted on the resemblance matrix to evaluate similarities between communities across surveyed sites (Figure 31). Combinations of sites (visualized as nodes) had community similarities ranging from 2.30-78.09%. The combination which had 78.09% similarity consisted of two sites, SUB-01 and NRB-07, where the former did not have AST presence detected and the latter did, though surveys were limited to a single year at SUB-01 and in near-drought conditions. However, 11 of 18 sites with AST presence clustered at a single node with 23.52% similarity in community structure and sub-combinations as similar as 56.29%.

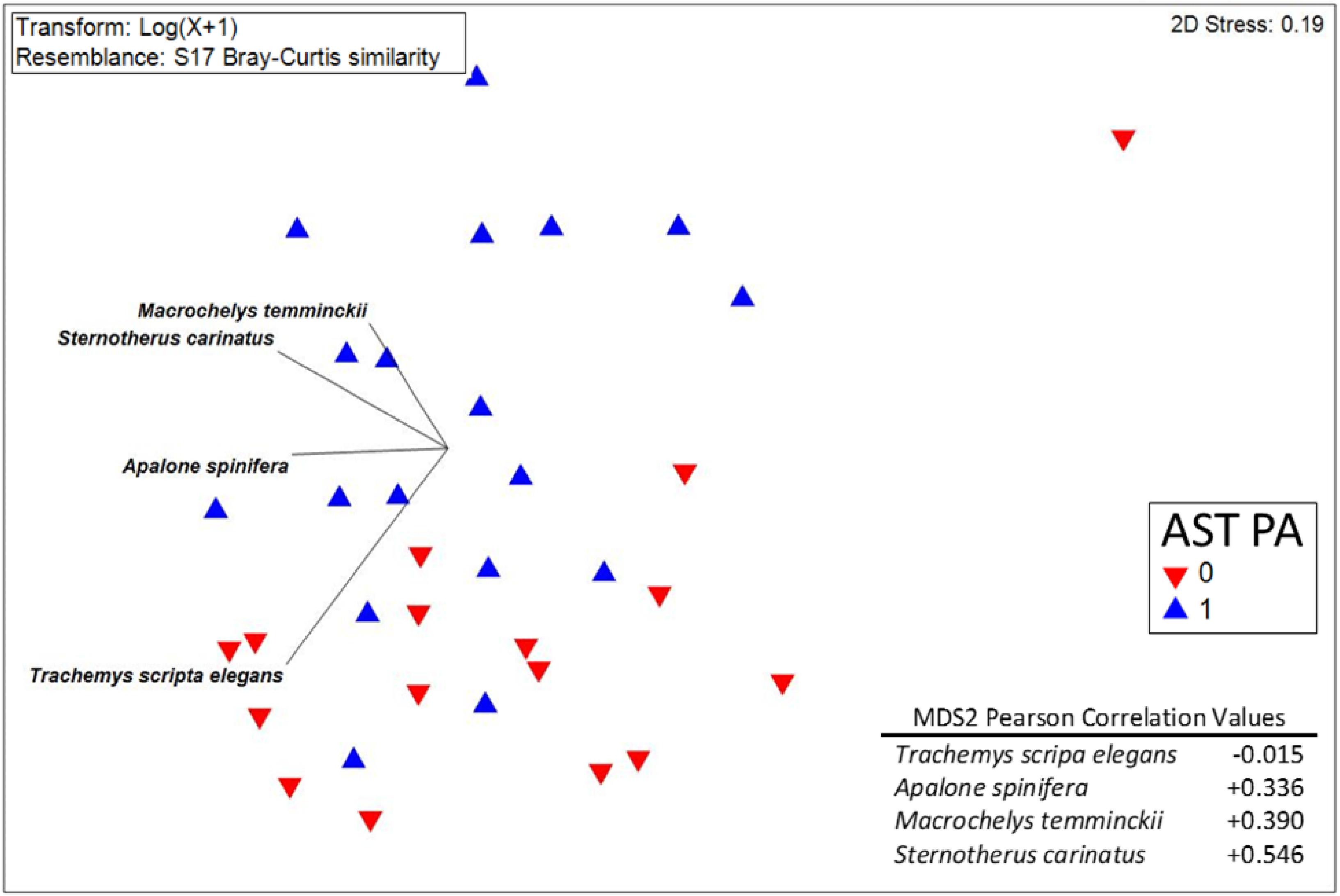


Figure 30 Non-metric multidimensional scaling (NMDS) plot of vertebrate community catch per unit effort (CPUE; number of individuals per trap night) from the current study. Sites are labeled by upward pointing blue triangles if Alligator Snapping Turtles (AST; *Macrochelys temminckii*) were captured, and downward pointing red triangles if ASTs were not captured. Ordinations and Pearson Correlation values are included for the four species with the highest correlation to MDS2: *Macrochelys temminckii*, *Sternotherus carinatus*, *Apalone spinifera*, and *Trachemys scripta elegans*. Species spatially distant and occurring in a different direction from the origin (e.g., *T. s. elegans*) were negatively correlated with AST presence.

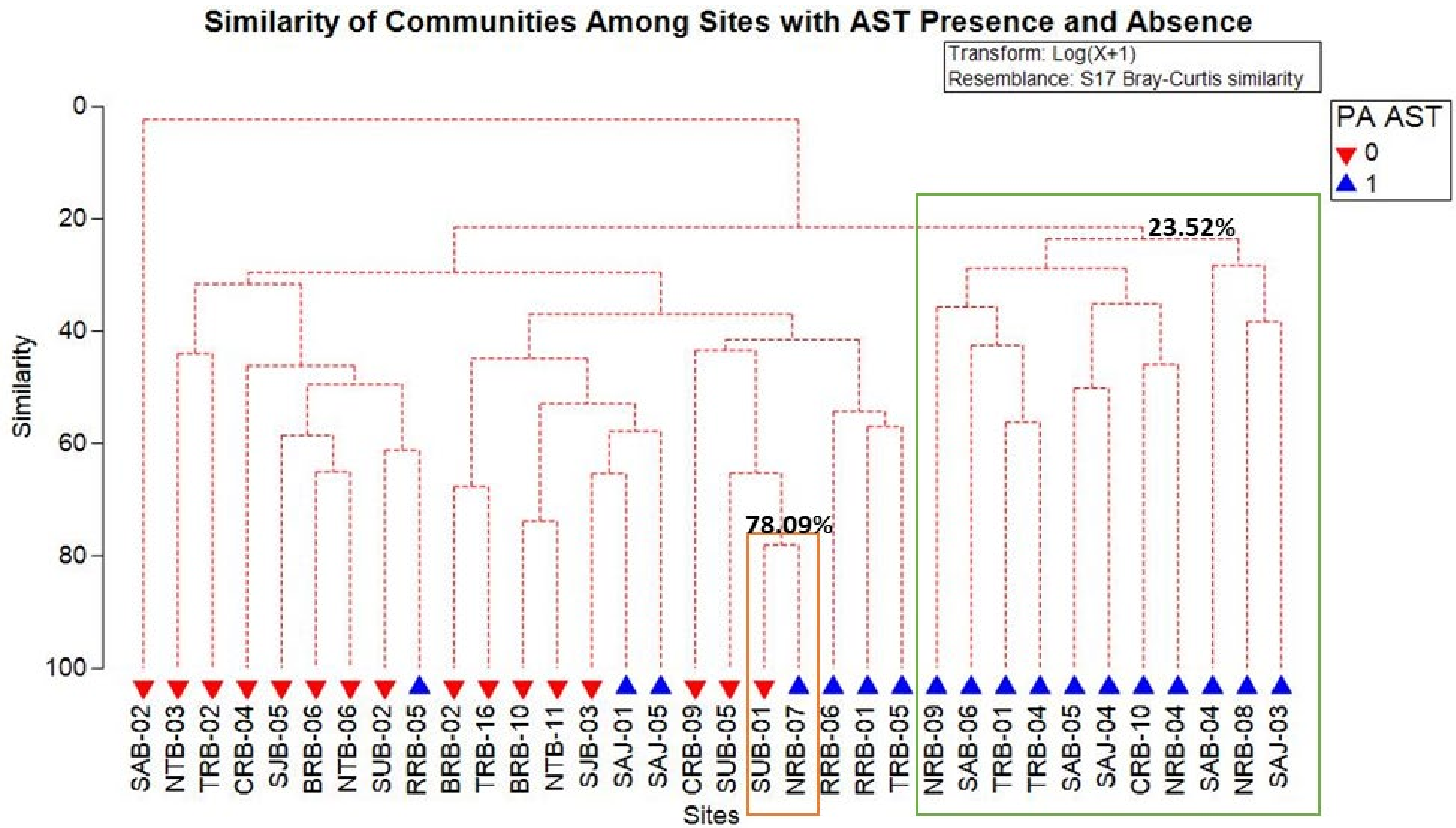


Figure 31 Similarity dendrogram resulting from cluster analysis of site resemblance data illustrating the similarity of community structures between sites where Alligator Snapping Turtle (AST; *Macrochelys temminckii*) presence was detected (red upward facing arrows) and not detected (blue downward facing arrows). The green box contains a cluster of 11 of 19 sites where AST presence combined at a single node with 23.52% similarity, and the orange box contains the site combination with the most similar community (78.09%).

Site Selection Matrix

Across all field sites (n = 34) we detected significantly higher site selection matrix scores for sites with AST captures (n = 18; median = 15) versus sites without AST captures (n = 16; median = 9) when scores related to the associated historic account(s) from the site selection process were considered (H = 10.687, df = 1, p = 0.001). However, exclusion of scores related to historic AST account scores did not produce significant differences between median values for sites with AST captures (n = 18; median = 9) versus those without AST captures (n = 16; median = 8). When applying score ranges to sites selected for this study, the majority of sites with positive AST detections (56%) fell into the "high" likelihood category while the majority of sites without positive detections (50%) fell into the "low" likelihood category. Scores of historical sites with verified AST observation(s) (n = 311) were similar to sites resulting in positive AST detections with the majority of scores (57%) falling into the high likelihood category. When testing proposed high, moderate, and low categories, a significant difference was detected (H = 10.638, df = 2, p = 0.005) assigned "High" (scores > 14) having significantly increased probability of predicting AST presence than those assigned "Low" (scores < 9). Sites with score values of "Moderate" (scores 9 ≤ 14) were not good predictors of AST presence or absence.

DISCUSSION

Historic CPUE rates and seasonality of Alligator Snapping Turtles in Texas

A primary goal of our study was to expand survey efforts to areas outside those already established in previous assessments (Appendix F). Multiple sampling locations were selected based on presence of spatial data from non-LEK and LEK sources. In areas where spatial data or historic reports were lacking, we identified candidate location using habitat assessment from aerial imagery. Sites selected based on non-LEK and LEK sources had the greatest success in confirming AST occupancy, while aerial habitat assessment and desktop reconnaissance alone were not a good predictor of occupancy. Though AST are thought to occur within the Brazos, Neches-Trinity, and San Jacinto-Brazos River basins of Texas, limited information exists to support current records from these basins (Dixon 2013, Hibbitts and Hibbitts 2016, USFWS 2021, Rosenbaum et al. 2022). Of the eight sites selected within these basins, two were selected based on LEK reports, though neither report had photographic evidence to support them. The remaining sites within these basins were selected solely based on aerial habitat composition and accessibility. We did not capture any ASTs within these basins, concurrent with previous studies that have evaluated these regions (Rudolph et al. 2002; Rosenbaum et al. 2022). Our results support previous studies with other focal species in that inclusion of LEK, or better yet, combination of LEK with other presence confirmation methods, ultimately increases the probability of detecting the target species at a given location.

Though the majority of previous surveys in Texas have focused on single visit to a site, false negative detections (e.g., no captures during trapping events at sites where AST have previously been captured), have been documented more recently (Munscher et. al 2020b, 2023; Rosenbaum et al. 2022; C. Franklin and V. Ricardez *unpubl. data*; current study). Additionally, detection success may depend on specific gear type and trapping methods, especially within lacustrine environments (Gulette et al. 2019; Mali et al. 2014; McKnight et al. 2015; Ream and Ream 1966; Riedle et al. 2015, 2016). Previous AST surveys in Texas have traditionally been conducted using 1.2 m hoop nets, though fyke nets and smaller diameter hoop nets are also successful (Gordon *unpubl. data*; Riedle et al. 2015, 2016). Studies utilizing homogenous sampling methods, such as a single trap type, may inaccurately estimate population data (Ream and Ream 1966, Tesche and Hodges 2015). We recommend future assessments focus on multiple trap or net sizes in order to maximize detection of AST, especially in middling or lower size classes, and re-evaluate effective trapping or capture methods within lacustrine and deep-water habitats (e.g., > 1 m depth).

Number of individuals captured, effort, and CPUE did not differ between this study and previous assessments of ASTs in Texas, with the exception of CPUE in the fall/winter season and when compared to CPUE rates from reports dating 4-9 years ago. During the fall/winter season of both years for the current survey, we experienced record flooding and near-record drought conditions, respectively. This temporal variation in environmental conditions likely played a role in our overall low CPUE rates for that season. Rosenbaum et al. (2022) conducted surveys at sites previously sampled by Rudolph et al. (2002) and found a greater than two-fold overall increase in CPUE between the two surveys. They considered it unlikely that the increase in CPUE was due to overall increases in population size and attributed it more to improvements in efficacy of trapping protocols and repeated sampling events at sites. Conversely, Munscher et al. (2020a, 2023) conducted targeted sampling within a restricted spatial area in Harris county and found similar CPUE rates between all years, though these CPUE rates were significantly higher than older (> 10 years) and more recent (< 3 years) surveys. Restrictions in spatial area and frequency of sampling (near monthly when compared to seasonally or annually as with other recent studies), may lead to increased likelihood of capture within this system in Harris county, though without comparable survey data across the ASTs range in east Texas during this time period, we are unable to determine if this is truly a representation of increased population size within 4-9 years ago. Regardless, because we conducted trapping efforts similarly to these previous assessments, found no significant differences in overall CPUE between surveys, and purposely expanded survey efforts to areas outside of previously established sampling locations, we believe our CPUE rates to be representative of expected CPUE rates for east Texas, as a whole.

Capture data from historic trapping surveys suggest that ASTs may be encountered more in spring (February–May) and fall/winter (October–January) seasons, though study design for previous surveys (e.g., lack of repeat site visits) may complicate temporal analyses. Data from LEK derived sources suggest that ASTs are more frequently encountered in spring and summer months, with limited observations reported in the fall/winter season. This increase in summer LEK observations may be an artifact of increased anthropogenic activity, especially in relation to recreational fishing. A review of the TPWD annual creel survey data to evaluate recreational *I. furcatus* (Blue Catfish) and *I. punctatus* catch rates found that spring and summer months represent peak-angling season (Nisbet et al. 2021). With increases in shore and water based recreational angling activities, the likelihood of recreational fisherman to encounter an AST is increased, especially as by-catch when targeting a fish species that may be considered an attractive prey source for AST, like *I. punctatus* or *I. furcatus* (Pritchard 1978). In the context of this study, increased number of AST encounters using LEK-derived data during summer months may not be as indicative of activity or population size, but more-so an indicator for when ASTs are actively or passively foraging.

Population estimates require long-term mark and recapture analysis. While the Lincoln-Person estimator can be modified to handle small samples sizes, the basic assumptions of this estimator does not render it the most useful for open populations (Seber 1982). Models utilizing the POPAN calculation (e.g., Jolly-Seber) can account for new unmarked animals that might not have been captured but require more robust sampling than was possible under the spatio-temporal constraints of this project (Schwarz and Arnason 1996; Munscher et al. 2023). A recent study by Rosenbaum et al. (2022) utilized multinomial N-mixture models to calculate expected abundance by site. When modeling with heterogeneity, the model returned an estimate of 7.57 AST per sample location, while estimates calculated by site resulted in slightly higher average of 8.75 turtles per location. Rosenbaum et al. (2022) did not report the average reach length for their sites or sampling events, so we were unable to convert site specific population estimates to estimated density per river kilometer. Conversely, in an ongoing long-term monitoring study (currently in its eighth year) conducted by Munscher et al. (2023) in Buffalo Bayou, a highly urbanized and constrained system, super-population estimates from Jolly-Seber models estimate a preliminary population size of 173 turtles across 39 r-km (approximately 4.44 turtles per r-km). Due to low sample size and recapture rates in the current study, we were unable to run a population estimate but were able to calculate average density of ASTs per r-km of 0.772 turtles across all sites and an average of 2.003 turtles per r-km at sites known to be occupied by AST. A key difference between the current and previous studies is that we specifically targeted sampling locations which had not been previously sampled for AST and we expanded efforts into areas where AST occupancy was unknown. It should be noted that duration of effort for collection of new data between the current study and the study conducted by Rosenbaum et al. (2022) was generally the same (approximately two years for each study), though Rosenbaum et al. also sampled locations with historic capture data collected by Rudolph et al. (2002). In general, large scale sampling and associated capture rates does allow for the identification of where AST occur and where to focus future sampling efforts for obtaining more robust demographic data for the species in Texas. We recommend that future surveys aim to specifically address answering questions related to population estimates over a wider and longer spatio-temporal scale.

Population structure for Alligator Snapping Turtles in Texas

Texas AST are characterized by low effective population sizes and strong population differentiation and it is likely that this population structure has existed for millions of years, and is rarely eroded naturally (Roman et al. 1999; Thomas et al. 2014, Rosenbaum et al. 2022, Munscher et al. 2023). We found that AST belong to three distinct genetic populations, each of which correspond to rivers that share a bay on the Gulf of Mexico. That populations are structured by rivers sharing a bay may be an artifact of those rivers being connected as a single drainage when sea levels were lower in the Pliocene and Pleistocene, as has been suggested before for AST and other Gulf Coast freshwater turtles (Lamb et al. 1994; Roman et al. 1999). Regardless of the cause for divergence, across the range of AST (Echelle et al. 2010; Apodaca et al. 2023), we find strong patterns of population subdivision (as defined by Hartl and Clark 1997), likely indicating that migration between populations is very low. We attest this divergence to rare overland dispersal (Reed et al. 2002). Still, some localities, especially in the Trinity and San Jacinto basins, did show admixture. Given the large geographic distances between the Red and San Jacinto drainages, and proximity to the Houston metropolitan area, we suspect that some of this admixture may be due to human movement. However, other areas, as in Turtle Bayou, may represent natural migration events, either through brackish bays, or via connected wetlands in neighboring drainages (D. Rosenbaum, *personal communication*).

Both at the river-drainage, and metapopulation level, Texas AST show low effective population sizes (e.g., effective number of individuals contributing to the genetic population; N_e) across the state. For example, despite sampling 40 individuals in a drainage over 18,000 square miles in area, we calculate that the Trinity River basin-population of AST has the evolutionary equivalent of a population with only 11 individuals (see Appendix C). This low effective population size puts this basin-population, and potentially others around the state, at risk from disease, inbreeding, and climate change. It is difficult to make comparisons of the estimated effective population size from this study for a few reasons. Here, we present the first determination of an effective population size at any level for AST, making comparisons to previous genetic assessments for AST impossible. Additionally, comparisons to other species are not useful in that other species likely differ in historical population structure and natural or life history traits. Furthermore, the methods used in other studies to calculate effective population size and the genetic markers used can impact overall determination of effective population size. Regardless, an effective population size under 50 has been suggested as a cutoff to list species as critically endangered by the IUCN because species with this low number of effective individuals are likely to face irreversible loss of genetic diversity (Garner et al. 2020). Although the average effective population size across all metapopulations established in this study is over the N_e = 50 threshold established by the IUCN (average N_e across all metapopulations = 214.9), the San Jacinto+Trinity metapopulation was calculated to be under this threshold (N_e = 25.9). Thus, it is possible that one or more of the Texas AST metapopulations will be vulnerable to the problems that arise from a lack of genetic diversity (e.g., increased disease susceptibility, lower fitness, less adaptive potential, etc.). Further studies, specifically within the San Jacinto+Trinity metapopulation, may be necessary to further refine or evaluate effective population size for AST populations in Texas, as a whole.

Despite low effective population sizes, and thus low overall genetic diversity, or possibly because of them, we did not detect an effect of dams on population subdivision. It is possible that low genetic diversity means that all localities within a drainage are too genetically homogenous to differentiate at such small scales. Models in some freshwater fish species indicate that genetic divergence would be expected to be visible after 40-60 generations (Ruzich et al. 2019), which equates to at least 440 years in AST (Dobie 1971). We also attempted to discover loci that could be used to sex AST, but were unsuccessful. This is important because juveniles, small males, and females may look very similar and be difficult to sex using external characteristics, as displayed by our age and size-class matrix testing. Identifying sex-linked loci to use genetic sexing could therefore be helpful in understanding population sex ratios, sex-biased dispersal and other important population traits. Identifying sex-linked loci using 3RAD can be difficult, as 3RAD randomly shears the genome. Given the stochastic nature of 3RAD sequencing, parts of the genome will be excluded by chance. Alternatively, given that only females carry the W chromosome, we may not have sequenced enough females to have W-linked loci carry through the analysis. Finally, given that the program recommends more than 100 sexed individuals without population structure to properly assign sex-linked loci, we may not have had enough power to distinguish the loci that are present in our dataset. Future assessments should attempt other analyses to try to target sex-linked loci in the future or, alternatively, add more individuals with known sexes from a given drainage to increase power to detect sex-linked loci in the future.

There are several important implications of these population genetics analyses for management of AST. First, overall genetic diversity and effective population sizes are low, so every effort should be made to retain connectivity among suitable habitat patches within all drainages occupied by AST. Effort should be made to document any abnormalities or die offs that may occur as a result of disease, or inbreeding, both of which are considerable risks to populations with low genetic diversity. Second, given the strong population structure, every effort should be made to translocate or repatriate confiscated AST to their correct river of origin as the likelihood of increasing genetic diversity naturally through mutations increases with population size.

Morphometric and demographic analyses of Texas Alligator Snapping Turtles

Morphometric data were consistent with known trends for the species (Pritchard 1989; Nelson 1999; Rudolph et al. 2002; Riedle 2014; Munscher et al. 2020a, 2023; Rosenbaum et al. 2022), hence our ability to combine data (mid-SCL and mass) compiled in the current study with that of previous assessments. Males were larger than females in all measurements and juveniles were significantly smaller than males or females. As resource managers make plans for future conservation of the species, especially for measures targeted as specific age classes (e.g., hatchlings, juveniles, sub-adults, and adults), we strongly recommend establishment of a defined age-size class structure for the species. These types of structure matrices are well established for highly conserved turtle species, especially sea turtles (Chaloupka and Limpus 2005). Turtles of differing size classes, and therefore associated age classes, are exposed to differing environmental and habitat conditions which may ultimately affect growth rates, survivorship, or reproductive development, especially at smaller (e.g., younger) sizes (or ages) (Avens et al. 2012). For example, body condition index (BCI) was highly correlated amongst AST in Texas, as a whole, but when we evaluated BCI using our proposed age-size class structure, we determined significant differences in BCI not only between sexes, but also between age classes (e.g., life history stages).

Overall, mean age and size class assignments in the field followed closely to those developed in our proposed matrix based on the existing literature. For example, mean size for sub-adults when age class was assigned in the field was 334.5 mm while the lower limit of our proposed adult size classification was 320 mm; a difference of 14.5 mm. Similarly, mean size for juveniles when age class was assigned in the field was 240.3 mm when the upper limit of the juvenile size classification was 249 mm, a difference of < 10 mm. For females confirmed by observation of reproductive structures using ultrasonography, the smallest individual in which follicles were observed was 348 mm mid-SCL (pre-cloacal tail length = 90 mm) while the largest was 484 mm mid-SCL (pre-cloacal tail length = 114 mm). When comparing mid-SCL to pre-cloacal tail length, a commonly used primary sexing technique for sex determination in the field, we found that males and females began externally and visibly differentiating near 350 mm mid-SCL and 80 mm pre-cloacal tail length. Taking all of these observations into consideration, a revision of the proposed age-size classification for adults to > 350 mm may be more prudent, though more data are needed across the species range to ensure there is no spatial variation in these values amongst populations outside of Texas.

The USFWS has identified a conservation need to assess "spatial variation in demographic rates related to reproduction, recruitment, and survival". Variation in reproduction, recruitment, and survival can be impactful at each life history stage of a given species, and thus, requires evaluation within each stage. Without an established age-size class structure (or similar) for the species, making broad conservation measures for individuals (or populations) of all sizes and ages may not be as beneficial as intended and, in some cases, may ultimately negatively impact individuals of a given age or size class. While the literature used to develop our proposed age-size class structure is not an exhaustive representation of the available information, it covers a wide range of AST populations throughout their historic range in the United States. We believe this is a reliable proposed age-class structure that is representative of AST populations as a whole, though further evaluation will be necessary in order to finely tune the cut off determined for each age class. Use of secondary (or even tertiary) sexing techniques will be imperative to final age-size class determinations, especially for individuals of middling or smaller body sizes (mid-SCL < 350 mm). As more data are collected for AST morphometrics throughout the species range (Rostal et al. 2023a), clearly defined age-size class determinations can be made on a range-wide level. Though we can only compare the proposed age-size class structure matrix to individuals captured in Texas, the western-most edge of the species range, we recommend that further evaluation and categorical assignments of this structure matrix be refined prior to imposing age and size class restrictive conservation measures in the future.

External injuries or abnormalities have been rarely documented in Texas AST populations. Incidence of shell algae was common (78.8% of turtles documented) and leeches were observed in half of the individuals captured. The potential effects of shell algae and leeches to AST fitness are unknown, but epiphytic (or “periphytic”, “epibiont”) growth has been documented in other freshwater and marine turtle populations (Ryan and Lambert 2005, McCoy et al. 2007, Kaleli et al. 2020, Roubeix et al. 2021). Major physical injuries (e.g., missing limbs, tail damage, scarring from previous wounds, etc.) were most frequently observed in males, though general shell damage (e.g. pock-marks, scratches on the carapace, etc.) were documented in all sexes. Though we are unable to speak to the magnitude at which these injuries or abnormalities affect the greater population in Texas, the correlation between these observations and overall abundance across individuals observed suggests that further evaluation may be needed to see if they are ultimately impactful to survivorship of the species, as a whole. We recommend that future assessments of AST include documentation of these traits, especially within different age classes, and make efforts to evaluate the overall impact to survivorship and health of the individuals.

To our knowledge, we have compiled the first documentation of reproductive development in wild-captured female AST in Texas. Though data are limited, an apparent lack of eggs or large follicles in the spring (April through June) and late summer (August through October) and an increase in follicular diameter from August through November correlates with similar findings in other states (Teare 2010, Thompson 2013, Rostal et al. 2023b). Though it is difficult to make extrapolations from the limited dataset, presence of a quiescent females during the fall/winter (e.g., during the period of follicular development) suggests that females in Texas may skip nesting seasons, similar to those observed in Louisiana (Dobie 1971). This could have major implications on the overall conservation of the species in that, if females are producing fewer clutches than originally expected, production and survivorship of hatchlings may also be reduced, overall. We failed to detect fully shelled eggs in any of the females assessed during the current study, but presence of quiescent females during what is typically considered the nesting season (April through June) suggests that these females may have recently deposited their clutch(es) prior to evaluation using ultrasonography. Additionally, observation of atretic follicles (e.g., follicles being reabsorbed) during the same period further confirms this theory. Previous studies in other states have identified nesting “season” windows to be short in duration (15-22 days) (Ewert and Jackson 1994, Jackson and Ewert 2023, Holcomb and Carr 2023). Should Texas ASTs exhibit short duration windows as in other states, the likelihood of our “missing” the window of shelled egg visualization is increased. More recently, though, the earliest and latest observed AST populations outside of the Apalachicola unit (USFWS 2021) occurred between 11 April and 2 August, respectively (Carr et al. 2023). In order to ultimately determine reproductive success and fecundity for AST in Texas, a full-scale evaluation of reproductive development, nesting, and hatchling success is necessary.

Habitat and community assemblages for Texas Alligator Snapping Turtles

We found that evaluation of environmental and habitat variables at the site-specific level provided the most significant results for probability of detecting AST. While we evaluated environmental and habitat variables in a myriad of ways (e.g., by trap, by event, by basin, by metapopulation, etc.), summarizing variables by site was, overall, most useful. During the course of the two-year study, we experienced record-breaking flood conditions in year one and near-drought conditions in year two. This short-term temporal variation in environmental and habitat conditions likely affected our ability to evaluate associations at a finer (or, conversely, more large-scale) resolution, but, this overall variation in conditions is also representative of the range of conditions experienced by this long-lived species on a long-term temporal (and spatial) scale. We found that likelihood of detection was increased in areas where dissolved oxygen concentrations were near 10 mg/L, thalweg depth was > 2-meters, water temperatures were lower overall (mean = 23.31°C), in-water cover not associated with large woody debris or structure was increased, areas where substrate was primarily composed of fine materials, and in waterbodies with increased bank slope.

Riverine habitats, specifically those surrounded by forested riparian structure, also had a higher proportion of detection. These associations follow similarly to those recently documented by Rosenbaum et al. (2022) and previously established by Riedle et al. (2005). Though we sampled riverine and lacustrine habitats, our sample size for number of lacustrine habitats was overall low (11.8% of sites). Riverine sites with high sinuosity often allowed us to easily set traps in shallow areas on the inside of turns to target deeper water and undercut banks downstream, which ultimately may have skewed our data when compared to sites with deeper habitat. In riverine habitats, channelization, sinuosity, and overall restrictions in spatial distribution due to increased topography along banks forces individuals to identify microhabitat within the system for shelter, foraging, etc. Conversely, in ponds, lakes, and reservoirs, overall ability for dispersal and movements is greatly increased, thus likely lowering our overall ability to pinpoint exact microhabitats that may be selected for. In many instances resulting from our LEK data compilation, reporters observed AST within lacustrine habitats, opportunistically emerging to chase fish that may have been caught on hook and line or breeching the surface to breath. Additionally, reports to the LNVA mapper included chance observations of AST while agency representatives were conducting routine monitoring efforts in lacustrine habitats. Further evaluation of detection techniques beyond traditional trapping surveys may be necessary to identify areas within lacustrine habitats that may be preferred or utilized by AST in Texas.

Anthropogenic data analysis resulted in no significant differences in CPUE between the presence categories of fishing gear or access points. Though we did not detect significant interactions between CPUE and presence of fishing gear or access points, a recent study documented similar results. Lack of correlation between anthropogenic data and AST detection or abundance follows similarly to the human accessibility index (HAI) developed by Rosenbaum et al. (2022). Though they evaluated observed anthropogenic factors (e.g., trotlines, access points, etc.), they did not report a significant correlation between this HAI to AST occupancy or detection. Future assessment of the overall effects of active, passive, or derelict fishing gear types, access points, or other anthropogenic disturbances is necessary before final determinations can be made about the ultimate impact to AST populations in Texas, as a whole.

Here, we document a new protocol designed to detect metallic foreign bodies in wild-captured AST without having to expose individuals (turtles and humans alike) to potentially detrimental radiation, conduct time- and physically-intensive field assessments, or transport individuals from their location of origin for assessment. While we were only able to preliminary implement this protocol in a small number of individuals observed during the current study, we were successful in identifying presence of metallic foreign bodies in nearly 11% of wild-caught individuals using a handheld metal detector. Overall, our detection rates were lower than anticipated, though fell within ranges previously established for other freshwater turtle species (0-33% of individuals depending on species) (Steen et al. 2014). Our attempts at identifying the specific structure of internal metallic bodies using ultrasonography were unsuccessful, but in some individuals, we were able to confirm that fishing hooks (within the mouth) could be detected using this newly developed protocol. Further evaluation of the success, efficiency, and efficacy of this metal detection protocol will be beneficial to resource managers, as one of the key concerns for AST in particular is that of anthropogenic impacts from active, passive, or derelict fishing pressure on overall population success. Use of this protocol to confirm presence (or absence) of metallic foreign bodies within individual AST can be paired with observed anthropogenic influences (especially in relation to fishing pressure) for better evaluation of impacts to the species, overall. We recommend that future studies continue evaluation of efficiency and efficacy of this protocol, especially in relation to the greater AST population throughout its range in the United States.

We evaluated association of vertebrate assemblages at sites where AST were detected versus those where AST were not detected. Overall, we found a positive association between AST and *Sternotherus carinatus* (Razor-backed Musk Turtle) and negative association with *Trachemys scripta elegans* (Red-eared Slider Turtle, RES). Similarly to AST, many *Sternotherus* species (including *S. carinatus*) prefer habitats with increased microhabitat availability, cover, and tannic to darker stained waters (Munscher et al. 2020b). These types of waters typically host more robust benthic prey availability, which is a key component of the diet for *S. carinatus* (E. Munscher, *personal communication*). While the importance of benthic macroinvertebrates in the diet of AST is relatively unknown, certain macroinvertebrate species may be indicative of AST presence, though more research is needed on this topic. In our study, a negative correlation with RES could be an artifact of the RES avoidance of traps already occupied by AST. Observations of RES with broken or crushed shells resulting from AST attacks while in the same trap have been documented by researchers in other studies (E. Munscher, *personal observation*). At the large scale, AST and RES have been positively associated as “generalist” species in previous assessments (Riedle et al. 2008). Conversely, at a smaller scale, a negative correlation between AST and most other turtle species has been documented previously (Riedle et al. 2015).

Of the top four impactful species to AST community structure, no fish species identified as prey sources were included. When evaluating overall assemblages between survey sites, we found high similarity (78.09%) between two locations: one where AST were detected and the other where AST were not detected. Though assemblages between these sites showed high similarity, at the site where AST were not detected, RES was the only species documented. Conversely, at the site where AST were documented, we also captured *S. carinatus*, *Chelydra serpentina* (Snapping Turtle), *Micropterus salmoides* (Largemouth Bass), and RES, though RES was by far the more abundant species present (n = 40 compared to a combined n = 7 for all other species, including AST). The high similarity between these two sites is likely impacted by the increased prevalence of RES, however, when we removed occurrences of RES, community structure completely changed and we were unable to discern direct relationships. This high, negative correlation between AST and RES is surprising, as RES are found almost universally throughout east Texas and are considered a generalist or opportunistic endemic species. We recommend further evaluation of associated community structures, especially for species which may evade capture using traditional trapping techniques (e.g., benthic macroinvertebrates, small fish, minnows, etc.) to evaluate the impacts of these assemblage correlations on the greater AST population in Texas and their predictability of AST presence or absence.

Priority sites, selection, and recommendations for future directions

To target areas of expansion for field surveys for AST in Texas, we recommend use of our site selection matrix to facilitate selection of candidate locations (Appendix A). When implementing this matrix, sites which have a combined maximum score of ≥ 15 should be prioritized. Although this matrix can be adjusted based on specific project goals, positively confirmed historic accounts are a major factor in determining likelihood of detection at a given site. While multiple studies have applied Multi-Criteria Decision-Making matrices (like our site selection matrix), the majority have focused on application for evaluation of environmental parameters (Javaheri et al. 2006, Haaren and Fthenakis 2011, Uyan 2014, Wang et al. 2016, Zoghi et al. 2017). Here, we show that a Multi-Criteria Decision-Making-based matrix has potential in wildlife or ecological surveys using historical accounts and categorizing score ranges into levels of suitability.

In addition to the site selection matrix, we proposed the following list of candidate sites as primary (top priority; n = 30) and secondary (next priority; n = 17) areas for continued evaluation of AST populations in Texas (Table 15). This list of recommended priority locations for future surveys is compiled from sites identified in the current study as primary or secondary candidate locations, results from Rosenbaum et al. (2022) resulting in recapture of individuals at sites previously monitored by Rudolph et al. (2002) or where AST were captured after multiple sampling attempts, and results from Munscher et al. (2023) where AST have been actively monitored for more than seven years in Harris county. We believe that these locations represent a wide distribution of localities throughout the species range in east Texas and that captured made during repeated survey efforts indicate that populations within these localities may be persistent, at least in the near-future. Due to conservation concerns for the species, especially in regards to poaching, we only include these locations at the county level. Should resource managers be interested in more specific spatial information related to these locations, we recommend reaching out to the respective corresponding authors for each source noted.

Table 15 List of recommended locations for future population assessments of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) based on historic and current surveys in east Texas. Site type determined based on results of multiple survey efforts in the same location resulting in capture of AST across multiple years (primary = best recommendation; secondary = next best recommendation). Duration represents the span of time (in years) that a given study or set of studies covers.

Source	County	Type	Duration
Rudolph et al. (2002); Rosenbaum et al. (2022)	Anderson	Primary	20+ years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Angelina	Primary	20+ years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Angelina/Nacogdoches	Primary	20+ years
Rosenbaum et al. (2022)	Camp	Primary	2 years
Rosenbaum et al. (2022)	Cass	Primary	2 years
Rosenbaum et al. (2022)	Chambers	Primary	2 years
Rosenbaum et al. (2022)	Cherokee	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Collin	Primary	20+ years
Current study	Harris	Primary	2 years
Current study	Harris	Primary	2 years
Munscher et al. (2023)	Harris	Primary	7 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Harrison	Primary	20+ years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Harrison	Primary	20+ years
Rosenbaum et al. (2022)	Hunt	Primary	2 years
Rosenbaum et al. (2022)	Jasper	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Leon	Primary	20+ years
Current study	Marion	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Nacogdoches	Primary	20+ years
Rosenbaum et al. (2022)	Navarro	Primary	2 years
Current study	Polk	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	San Jacinto	Primary	20+ years
Current study	San Jacinto	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Shelby	Primary	20+ years
Rosenbaum et al. (2022)	Trinity	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Tyler	Primary	20+ years
Rosenbaum et al. (2022)	Van Zandt	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Walker	Primary	20+ years
Rosenbaum et al. (2022)	Waller	Primary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Wood	Primary	20+ years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Wood	Primary	20+ years
Current study	Bowie	Secondary	2 years
Current study	Chambers	Secondary	2 years
Current study	Hardin/Jefferson	Secondary	2 years
Current study	Harris	Secondary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Houston	Secondary	20+ years
Current study	Hunt	Secondary	2 years
Current study	Jasper	Secondary	2 years
Rosenbaum et al. (2022)	Kaufman	Secondary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Liberty	Secondary	20+ years
Current study	Liberty	Secondary	2 years
Current study	Liberty	Secondary	2 years
Current study	Newton	Secondary	2 years
Current study	Orange	Secondary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	San Augustine	Secondary	20+ years
Current study	Shelby	Secondary	2 years
Rudolph et al. (2002); Rosenbaum et al. (2022)	Titus	Secondary	20+ years
Rosenbaum et al. (2022)	Upshur	Secondary	2 years

Final Conclusions

Here, we show that:

- Use of local ecological knowledge can be used to guide surveys focused on detection of cryptic or difficult to find species in areas where occupancy is previously unestablished.
- Overall, CPUE for AST populations in Texas have not changed in the past 10+ years, especially between surveys conducted > 10 and < 3 years ago.
- Overall, AST in Texas appear to be most active during the Spring (February–May) and Summer (June–September) seasons, though this may be due to anecdotal observations being correlated with increased recreational activity during those same time periods.
- Based on results of population genetic analyses, Texas AST can be divided into three distinct metapopulations including the Red, Cypress and Sulphur river basins (metapopulation #1), Sabine and Neches river basins (metapopulation #2), and San Jacinto and Trinity river basins (metapopulation #3).
- Effective population size and genetic diversity for AST in Texas are overall low, therefore effect of anthropogenic structures, such as dams, could not be assessed. Though it may take > 400 years before direct impacts of these structure types can be assessed, future studies are needed to determine overall impacts of these structures.
- Morphometric data were consistent with known trends and previous studies. Specifically, Body Condition Index was highly correlated among AST in Texas, though differed between sexes and proposed age-size classes.
- Establishment of a distinct age-size class matrix for the species is imperative for future conservation efforts focused on specific life history stages. We propose an age-size class structure that we believe accurately represents AST in Texas, though it should be further evaluated and refined to reflect the greater AST population in the United States.
- Further evaluation of the impacts of external injuries or abnormalities (e.g., epiphytic growth or external parasites) are recommended to assess impacts to survivorship of AST.
- To our knowledge, we have compiled the first documentation of reproductive development in wild-captured female AST in Texas. Observation of presence (or absence) of specific reproductive structures suggests that AST in Texas are nesting during the Spring (April–June) season, though females may not be clutching every year.
- Likelihood of AST detection was increased in areas where dissolved oxygen concentrations were near 10 mg/L, thalweg depth was > 2-meters, water temperatures were lower overall (mean = 23.31°C), in-water cover not associated with large woody debris or structure was increased, areas where substrate was primarily composed of fine materials, and in waterbodies with increased bank slope.
- Riverine habitats (especially those surrounded by forested riparian structure) had the highest proportion of AST detections, though further evaluation of microhabitat selection in lacustrine environments (specifically using less traditional techniques from hoop trap surveys) are needed.
- Anthropogenic data associated with active, passive, or derelict fishing gear observations and access points were not significantly correlated to AST presence or non-detection, similarly to recent studies in Texas. Further evaluation of the influence of anthropogenic disturbances or use of riverine and lacustrine habitats are needed.
- Implementation of a newly developed protocol for detection of foreign metallic objects using a handheld metal detector was successful in locating internal metallic objects, but unable to determine the type of object. This protocol confirmed that fishing hooks could

be detected using the handheld metal detector, but further evaluation of the protocols wider application and ability to identify specific metallic structures is needed.
- Application of our site selection matrix and re-assessment of our primary and secondary recommended survey locations will aid future efforts to evaluate questions related to the greater AST population (and metapopulations) in Texas.

Ultimately, while we were able to compile, compare, and add to the existing base of knowledge for AST in Texas, a full population viability assessment requires multiple years, even decades, in order to elucidate meaningful relationships, especially amongst different life history stages. Future efforts to continue long-term monitoring surveys of AST populations in Texas will be critical in the conservation, protection, and, stable population structure. Many questions still remain about impacts to AST, impacts from AST, and overall fluctuations in survivorship for all life history stages of AST, but here we have laid the groundwork for future efforts.

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Appendix A – Site Selection Matrix and Recommended Scoring Indices

Site selection matrix, scoring criteria, and recommended indices that was applied, tested, and is recommended for future assessments.

Appendix Table A.1 Final Site Selection Matrix developed to aid in future selection of new survey areas for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) populations in Texas.

Category	Scale	0	1	2	3	4	5
Reliability of sighting	0-5	No report of AST in area	Anecdotal report that "may be an AST", no photograph or physical voucher	Report from multiple anecdotal accounts though accuracy of ID may not be as reliable as a biologist, no photograph available	Report from biologist/reliable source able to confidently discern AST from other turtles; no photograph	Report verified by biologist/reliable source but photograph is blurry/difficult to confirm positive ID	Report verified via photographic evidence and confirmed by trained biologist(s); includes accounts from published reports, theses, dissertations, or journal articles
Quality of geographic data	0-3	No description of location	Location identified at macro scale (e.g. region, river basin, etc.); includes Rudolph et al. (2002) sites georeferenced from Map 2 in report and mail in surveys	Location identified in relation to specific point (e.g. near bridge crossing); includes iNaturalist obscured locations and VertNet locations where GPS coordinates vary from location description	Precise and verified GPS coordinates reported with no obscuring or alterations	NA	NA
Age of sighting	0-3	No date reported	> 10 years ago	4-9 years ago	Within last 3 years	NA	NA
Physical accessibility of site	0-3	Accessibility unknown or indeterminable	Steep (cliff-like) banks with large (<10ft) drop offs; unreasonable distance from access point; dense vegetation prohibits water access	Access difficult but possible based on desktop reconnaissance; may require field recon	Access available (boat ramp, etc.); proximity reasonable for work completion in a single day; field recon not required	NA	NA
Site characteristics (within 250 m)	0-3	Unknown	No in-water cover; high level of alteration (e.g. channelized, all in-water cover removed)	Mixed in-water and canopy cover over water; little/no snags; prey availability limited	Best habitat characteristics related to historic AST waterbody use	NA	NA
Tampering potential	0-2	Unknown	High likelihood of gear/equipment being tampered with	Site well protected with little/no threat of gear tampering	NA	NA	NA
Access permission obtainable	0-2	Landowner Unknown	Landowner known but not contacted	Access already granted or public	NA	NA	NA
Max score = 21							

Total Score	Priority	Description
< 9	**Low**	**Lowest priority for implementation of Alligator Snapping Turtle (*Macrochelys temminckii*) survey efforts**
9 – 14	**Moderate**	**Moderate priority for implementation of Alligator Snapping Turtle (*Macrochelys temminckii*) survey efforts**
> 14	**High**	**Highest priority for implementation of Alligator Snapping Turtle (*Macrochelys temminckii*) survey efforts**

Appendix B – Data Used for Historic Capture and CPUE Comparisons

Includes a list of publicly accessible historic data compiled and used for comparisons to the current study. Specific date ranges are provided whenever possible. Munscher et al. (2023) reported data collected between 2016-2018 as total number, effort, and CPUE by year, but provided more specific date ranges and values in Munscher et al. (2020a). Therefore, we retained the more specific data, when available, and only included updated data for years which more specific values were not available.

Appendix Table B.1 Sources of historic Alligator Snapping Turtle (AST, *Macrochelys temminckii*) capture numbers (*N*), effort (as number of trap nights), and catch per unit effort (CPUE, number of turtles per trap night) data used for comparisons with the current study. Includes waterbody name, county, metapopulation associated with the current study (SJ+T = San Jacinto+Trinity, Sa+N = Sabine+Neches, R+C+S = Red+Cypress+Sulphur), sampling date range, relative age of the report(s), and season associated with the current study.

Source	Waterbody	County	Meta	Date Range	Age	Season	# ASTs	Effort	CPUE
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	10/15/2013-10/16/2016	4-9 years	Fall/Winter	6	10	0.600
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	12/18/2016-12/20/2016	4-9 years	Fall/Winter	2	10	0.200
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	01/20/2017-01/22/2017	4-9 years	Fall/Winter	4	10	0.400
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	02/10/2017-02/12/2017	4-9 years	Spring	4	10	0.400
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	04/07/2017-04/09/2017	4-9 years	Spring	6	10	0.600
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	06/09/2017-06/11/2017	4-9 years	Summer	4	10	0.400
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	07/07/2017-07/09/2017	4-9 years	Summer	2	10	0.200
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	10/06/2017-10/08/2017	4-9 years	Fall/Winter	2	10	0.200
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	11/10/2017-11/12/2017	4-9 years	Fall/Winter	3	10	0.300
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	12/01/2017-12/03/2017	4-9 years	Fall/Winter	4	10	0.400
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	03/23/2018-03/25/2018	4-9 years	Spring	6	10	0.600
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	04/20/2018-04/22/2018	4-9 years	Spring	1	10	0.100
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	04/27/2018-04/29/2018	4-9 years	Spring	2	10	0.200
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	06/02/2018-06/06/2018	4-9 years	Summer	3	10	0.300
Munscher et al. (2020a)	Buffalo Bayou	Harris	SJ+T	10/112018/-10/13/2018	4-9 years	Fall/Winter	19	23	0.826
Munscher et al. (2023)	Buffalo Bayou	Harris	SJ+T	2019	4-9 years	Unknown	30	112	0.267
Munscher et al. (2023)	Buffalo Bayou	Harris	SJ+T	2020	< 3 years	Unknown	30	118	0.254
Munscher et al. (2023)	Buffalo Bayou	Harris	SJ+T	2021	< 3 years	Unknown	23	42	0.547
Nelson (1999)	Bingham Lake	Tyler	Sa+N	May 1996-Aug 1998	> 10 years	Unknown	66	NR	0.145
Riedle (2014)	Catfish Creek	Anderson	SJ+T	Apr 2006-Aug 2009	> 10 years	Unknown	13	1001	0.013
Riedle (2014)	Keechi Creek	Leon	SJ+T	Jun-Jul 2009	> 10 years	Unknown	3	37	0.081
Rosenbaum et al. (2022)	Catfish Creek	Anderson	SJ+T	2020-2021	< 3 years	Unknown	17	45	0.378
Rosenbaum et al. (2022)	Old Neches Lake	Angelina	Sa+N	2020-2021	< 3 years	Unknown	9	45	0.200
Rosenbaum et al. (2022)	Angelina River	Angelina/Nacogdoches	Sa+N	2020-2021	< 3 years	Unknown	1	45	0.022
Rosenbaum et al. (2022)	East Fork Trinity River	Collin	SJ+T	2020-2021	< 3 years	Unknown	1	45	0.022
Rosenbaum et al. (2022)	Harrison Bayou	Harrison	R+C+S	2020-2021	< 3 years	Unknown	5	45	0.111
Rosenbaum et al. (2022)	Caddo Lake	Harrison	R+C+S	2020-2021	< 3 years	Unknown	7	45	0.156
Rosenbaum et al. (2022)	Ratcliffe Lake	Houston	Sa+N	2020-2021	< 3 years	Unknown	16	45	0.356
Rosenbaum et al. (2022)	Keechi Creek	Leon	SJ+T	2020-2021	< 3 years	Unknown	13	45	0.289
Rosenbaum et al. (2022)	Bonaldo Creek	Nacogdoches	Sa+N	2020-2021	< 3 years	Unknown	15	65	0.231

Appendix Table B.1 Sources of historic Alligator Snapping Turtle (AST, *Macrochelys temminckii*) capture numbers (N), effort (as number of trap nights), and catch per unit effort (CPUE, number of turtles per trap night) data used for comparisons with the current study. Includes waterbody name, county, metapopulation associated with the current study (SJ+T = San Jacinto+Trinity, Sa+N = Sabine+Neches, R+C+S = Red+Cypress+Sulphur), sampling date range, relative age of the report(s), and season associated with the current study.

Source	Waterbody	County	Meta	Date Range	Age	Season	# ASTs	Effort	CPUE
Rosenbaum et al. (2022)	Ayish Bayou	San Augustine	Sa+N	2020-2021	< 3 years	Unknown	2	45	0.044
Rosenbaum et al. (2022)	East Fork San Jacinto River	San Jacinto	SJ+T	2020-2021	< 3 years	Unknown	3	45	0.067
Rosenbaum et al. (2022)	Swede Johnson Bayou	Shelby	Sa+N	2020-2021	< 3 years	Unknown	4	45	0.089
Rosenbaum et al. (2022)	White Oak Creek	Titus	R+C+S	2020-2021	< 3 years	Unknown	1	45	0.022
Rosenbaum et al. (2022)	Bingham Lake	Tyler	Sa+N	2020-2021	< 3 years	Unknown	11	45	0.244
Rosenbaum et al. (2022)	West Fork San Jacinto River	Walker	SJ+T	2020-2021	< 3 years	Unknown	2	45	0.044
Rosenbaum et al. (2022)	Lake Fork	Wood	Sa+N	2020-2021	< 3 years	Unknown	3	45	0.067
Rosenbaum et al. (2022)	Beaver Lake	Wood	Sa+N	2020-2021	< 3 years	Unknown	12	45	0.267
Rudolph et al. (2002)	Catfish Creek	Anderson	SJ+T	06/08/1999-06/10/1999	> 10 years	Summer	3	45	0.067
Rudolph et al. (2002)	Angelina River	Angelina/Nacogdoches	Sa+N	05/25/1999-05/27/1999	> 10 years	Spring	1	30	0.033
Rudolph et al. (2002)	Old Neches Lake	Angelina	Sa+N	05/15/2001-05/17/2001	> 10 years	Spring	5	45	0.111
Rudolph et al. (2002)	Bonaldo Creek	Nacogdoches	Sa+N	Jun-Jul 2000	> 10 years	Summer	5	55	0.091
Rudolph et al. (2002)	East Fork Trinity River	Collin	SJ+T	08/15/2001-08/17/2001	> 10 years	Summer	1	45	0.022
Rudolph et al. (2002)	Caddo Lake	Harrison	R+C+S	05/12/2000-05/14/2000	> 10 years	Spring	4	15	0.267
Rudolph et al. (2002)	Harrison Bayou	Harrison	R+C+S	05/12/2000-05/14/2000	> 10 years	Spring	3	30	0.100
Rudolph et al. (2002)	Keechi Creek	Leon	SJ+T	07/14/1999-07/16/1999	> 10 years	Summer	8	45	0.178
Rudolph et al. (2002)	Pickett's Creek	Liberty	SJ+T	07/25/2000-07/27/2000	> 10 years	Summer	1	45	0.022
Rudolph et al. (2002)	Lost Creek	Newton	Sa+N	10/19/2000-10/21/2000	> 10 years	Fall/Winter	2	45	0.040
Rudolph et al. (2002)	East Fork San Jacinto River	San Jacinto	SJ+T	09/27/2000-09/29/2000	> 10 years	Summer	1	45	0.022
Rudolph et al. (2002)	Swede Johnson Bayou	Shelby	Sa+N	08/17/2000-08/19/2000	> 10 years	Summer	4	45	0.089
Rudolph et al. (2002)	Bingham Lake	Tyler	Sa+N	08/24/2000-08/25/2000	> 10 years	Summer	2	72	0.028
Rudolph et al. (2002)	West Fork San Jacinto River	Walker	SJ+T	06/16/2001-06/18/2001	> 10 years	Summer	2	45	0.044
Rudolph et al. (2002)	Beaver Lake	Wood	Sa+N	07/20/1999-07/22/1999	> 10 years	Summer	4	45	0.089
Rudolph et al. (2002)	Lake Fork	Wood	Sa+N	06/13/2001-06/15/2001	< 3 years	Summer	2	45	0.044

Appendix B Literature Cited

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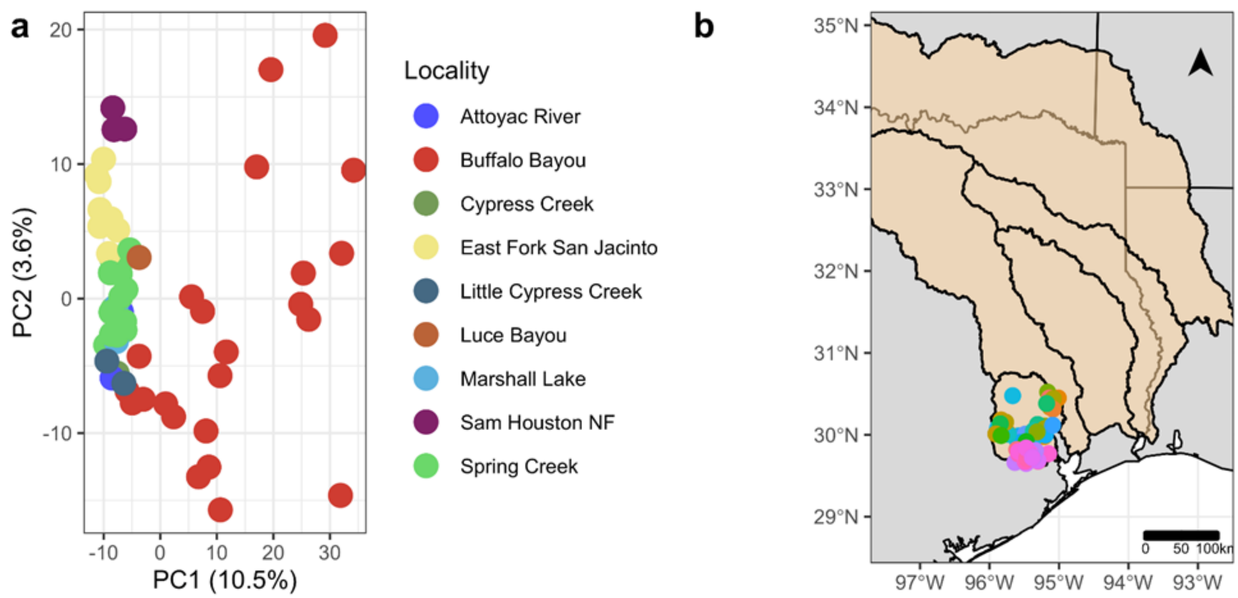
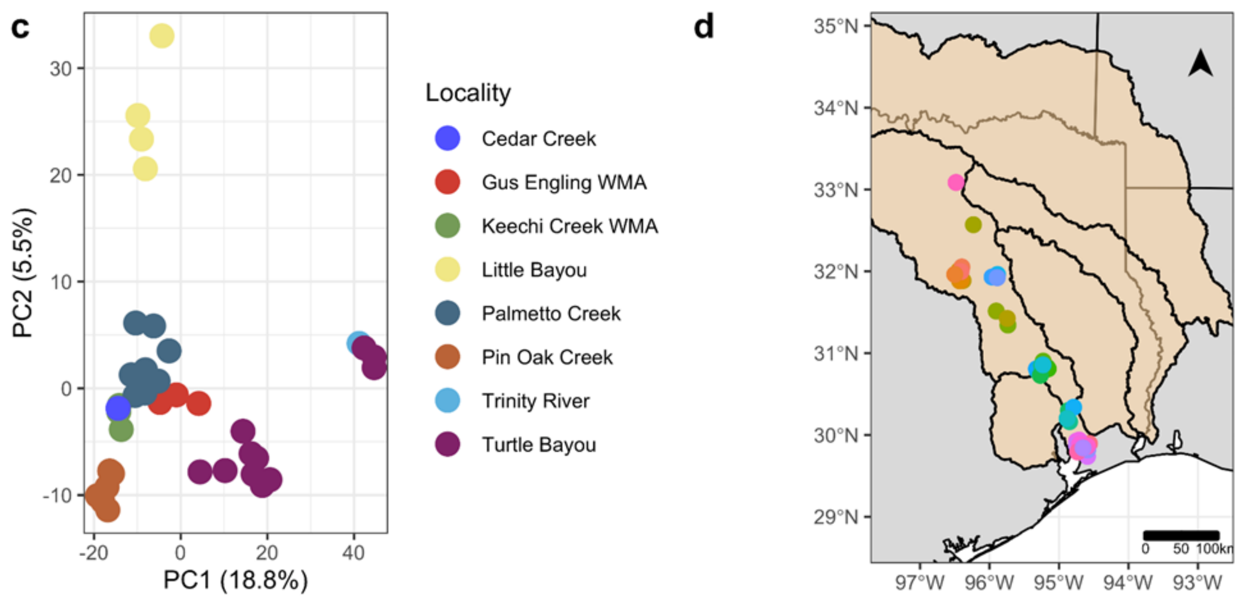
Appendix C – Basin-level Population Genetic Analyses

To better aid resources managers in Texas, we attempted to elucidate meaningful associations within the basin of origin for population genetic analyses. Overall, sample sizes were too low for us to evaluate meaningful relationships, but here we present the results of this basin-level evaluat, where possible.

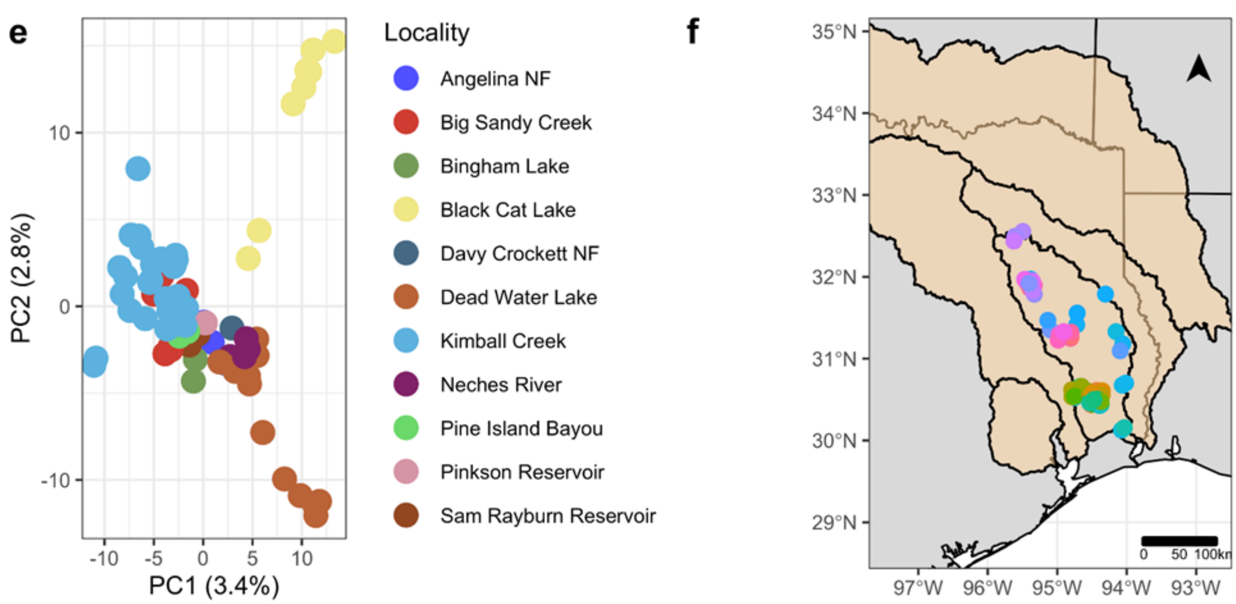
We repeated the PCA for each river drainage using identical parameters as in the metapopulation level analyses (Appendix Figures C.1 through C.5). We slightly jittered the locations of dots in the PCA maps to make as many of the dots visible as possible. Using the same dataset as in genetic diversity, population subdivision and effective population size analyses at the metapopulation level, we also calculated these statistics separately for each for location, and calculated F_{ST} for each locality within a river basin (Appendix Tables C.1 through C.7).



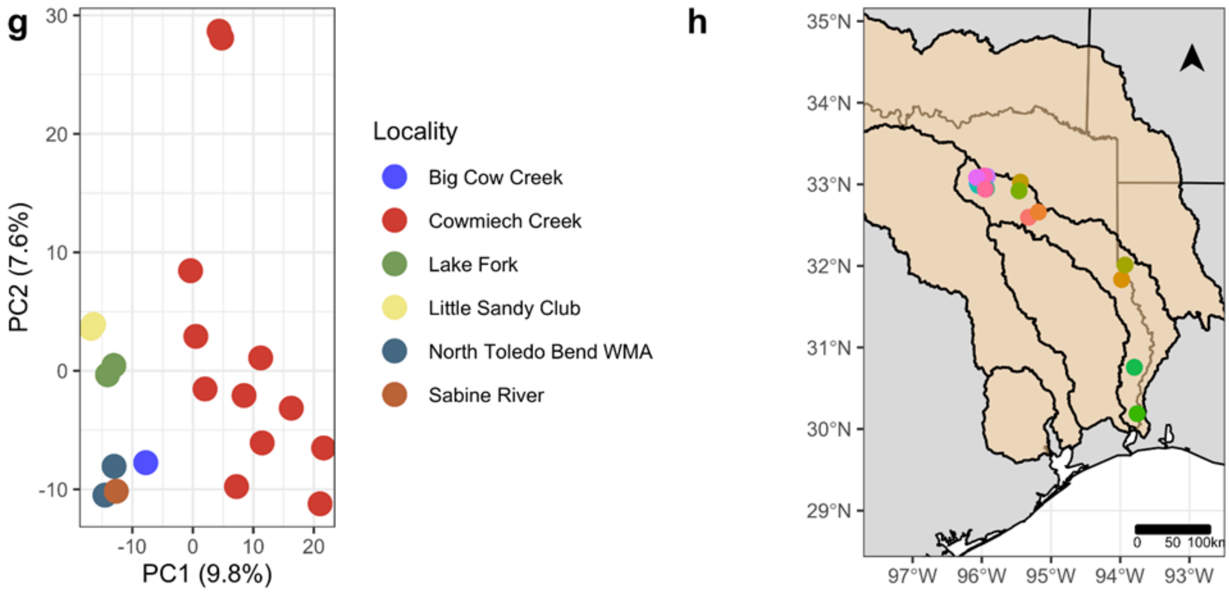
Appendix Figure C.1 Principal Component Analysis (PCA) of 60 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from the San Jacinto River basin in PC-space (a), and on a map (b). Overall, PCA is agnostic to population grouping. Individuals are colored by locality within the basin. Points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.



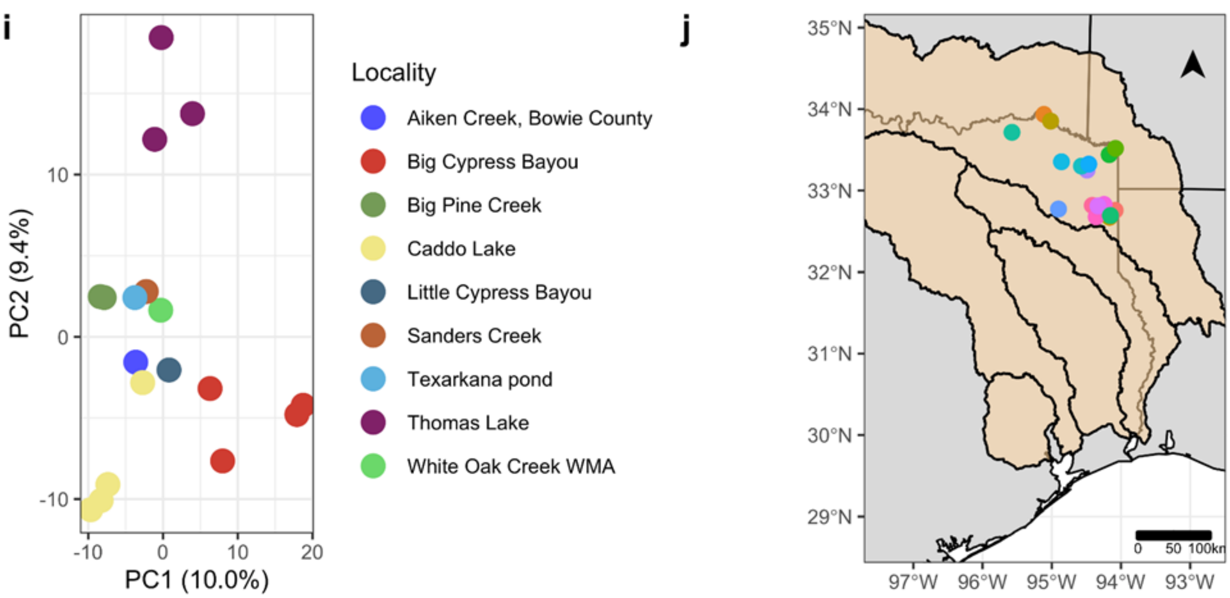
Appendix Figure C.2 Principal Component Analysis (PCA) of 40 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from the Trinity River basin in PC-space (c), and on a map (d). Overall, PCA is agnostic to population grouping. Individuals are colored by locality within the basin. Points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.



Appendix Figure C.3 Principal Component Analysis (PCA) of 77 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from the Neches River basin in PC-space (e), and on a map (f). Overall, PCA is agnostic to population grouping. Individuals are colored by locality within the basin. Points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.



Appendix Figure C.4 Principal Component Analysis (PCA) of 20 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from the Sabine River basin in PC-space (g), and on a map (h). Overall, PCA is agnostic to population grouping. Individuals are colored by locality within the basin. Points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.



Appendix Figure C.5 Principal Component Analysis (PCA) of 16 Alligator Snapping Turtles (AST; *Macrochelys temminckii*) from the Red River basin in PC-space (i), and on a map (j). Overall, PCA is agnostic to population grouping. Individuals are colored by locality within the basin. Points on the map are colored by three PC-axes (PC1 is mapped to red, PC2 is mapped to green, and PC3 is mapped to blue) with brighter colors indicating higher values on each axis.

Appendix Table C.1 Sample size (*n*), observed heterozygosity (H_O; Nei 1987), within-population gene diversity (sometimes referred to as expected heterozygosity, H_S; Nei 1987), within-population subdivision (F_{IS}; Nei 1987), and effective population size (N_e; Waples and Feutry 2021) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) by basin and locality in Texas. Due to samples being provided by multiple research groups, arbitrary reference numbers ("Ref. #") were assigned to each locality in order to pair population subdivision (F_{ST}) data in subsequent tables. Overall statistics for each major basin are italicized. Note that population-level statistics, especially H_S and F_{IS}, should be interpreted with caution when *n* < 3. NA = could not be calculated due to low sample size.

Basin and locality	Ref #	*n*	H_O	H_S	F_{IS}	N_e
Red River basin		*16*	*0.0696*	*0.0698*	*0.0038*	*444.4 (411.7 - 482.7)*
Pond Near Texarkana	Red-1	1	0.0764	NA	NA	
Aiken Creek, Bowie County	Red-2	1	0.0603	NA	NA	
Big Cypress Bayou, Caddo Lake	Red-3	4	0.0789	0.0700	-0.1262	
Big Pine Creek	Red-4	2	0.0561	0.0594	0.0553	
Caddo Lake	Red-5	3	0.0656	0.0598	-0.0971	
Little Cypress Bayou	Red-6	1	0.0714	NA	NA	
Thomas Lake, Sulphur River	Red-7	3	0.0705	0.0722	0.0237	
White Oak Creek WMA	Red-8	1	0.0336	NA	NA	
Neches River basin		*77*	*0.0882*	*0.0887*	*0.0059*	*211.6 (211.0 - 212.2)*
Angelina National Forest	Nec-01	2	0.0752	0.0660	-0.1402	
Big Sandy Creek	Nec-02	9	0.0893	0.0886	-0.0084	
Bingham Lake	Nec-03	2	0.0520	0.0451	-0.1542	
Black Cat Lake	Nec-04	8	0.0845	0.0804	-0.0517	
Davy Crockett National Forest	Nec-05	2	0.0651	0.0460	-0.4151	
Dead Water Lake	Nec-06	14	0.0859	0.0840	-0.0227	
Kimball Creek, Big Sandy Creek	Nec-07	31	0.0927	0.0903	-0.0268	
Neches	Nec-08	4	0.0770	0.0847	0.0908	
Pine Island Bayou	Nec-09	2	0.0875	0.0882	0.0075	
Pinkson Reservoir	Nec-10	1	0.0748	NA	NA	
Sam Rayburn Reservoir	Nec-11	2	0.0580	0.0357	-0.6262	
San Jacinto River basin		*60*	*0.0760*	*0.0786*	*0.0329*	*12.6 (12.6-12.6)*
Attoyac River	San-1	2	0.0623	0.0567	-0.1000	
Buffalo Bayou	San-2	24	0.0961	0.0971	0.0111	
Cypress Creek, W Fork San Jacinto	San-3	1	0.0639	NA	NA	
East Fork San Jacinto	San-4	8	0.0593	0.0576	-0.0299	
Little Cypress Creek, Spring Creek	San-5	2	0.0679	0.0572	-0.1868	
Luce Bayou	San-6	1	0.0735	NA	NA	
Marshall Lake	San-7	3	0.0644	0.0578	-0.1149	
Sam Houston National Forest	San-8	3	0.0507	0.0408	-0.2426	
Spring Creek	San-9	16	0.0641	0.0630	-0.0182	
Sabine River basin		*20*	*0.0838*	*0.0843*	*0.0063*	*43.3 (43.0 - 43.6)*
Big Cow Creek	Sab-1	1	0.0956	NA	NA	
Cowleech Creek	Sab-2	12	0.0834	0.0811	-0.0276	
Lake Fork	Sab-3	2	0.0661	0.0510	-0.2958	
Little Sandy Hunting and Fishing Club	Sab-4	2	0.0599	0.0407	-0.4717	
North Toledo Bend WMA	Sab-5	2	0.0670	0.0491	-0.3651	
Sabine River	Sab-6	1	0.0904	NA	NA	
Trinity River basin		*40*	*0.0806*	*0.0909*	*0.1138*	*11.0 (11.0 - 11.1)*
Cedar Creek	Tri-1	1	0.0594	NA	NA	
Gus Engling WMA	Tri-2	3	0.0664	0.0535	-0.2405	
Keechi Creek WMA	Tri-3	3	0.0532	0.0447	-0.1899	
Little Bayou	Tri-4	4	0.0822	0.0839	0.0213	
Palmetto Creek	Tri-5	10	0.0830	0.0768	-0.0802	
Pin Oak Creek	Tri-6	7	0.0631	0.0597	-0.0568	
Trinity	Tri-7	1	0.0381	NA	NA	
Turtle Bayou	Tri-8	11	0.0960	0.1019	0.0583	

Appendix Table C.2 Population subdivision (F_{ST}) by drainage of origin for Alligator Snapping Turtle (AST; *Macrochelys temminckii*) surveys in Texas as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Basin F_{ST} values	Neches	Red	Sabine	San Jacinto	Trinity
Neches	NA	0.3936	0.0833	0.368	0.2654
Red	0.3936	NA	0.4159	0.4811	0.4381
Sabine	0.0833	0.4159	NA	0.3864	0.2879
San Jacinto	0.368	0.4811	0.3864	NA	0.0722
Trinity	0.2654	0.4381	0.2879	0.0722	NA

Appendix Table C.3 Population subdivision (F_{ST}) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the Red River basin as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes and reference numbers (“Ref. #”) for each locality. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Locality F_{ST} values								
	Red-1	**Red-2**	**Red-3**	**Red-4**	**Red-5**	**Red-6**	**Red-7**	**Red-8**
Red-1	NA	NA	NA	NA	NA	NA	NA	NA
Red-2	NA	NA	NA	NA	NA	NA	NA	NA
Red-3	NA	NA	NA	0.0946	0.0295	NA	0.0327	NA
Red-4	NA	NA	0.0946	NA	0.0910	NA	0.052	NA
Red-5	NA	NA	0.0295	0.091	NA	NA	0.0167	NA
Red-6	NA	NA	NA	NA	NA	NA	NA	NA
Red-7	NA	NA	0.0327	0.052	0.0167	NA	NA	NA
Red-8	NA	NA	NA	NA	NA	NA	NA	NA

Appendix Table C.4 Population subdivision (F_{ST}) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the Neches River basin as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes and reference numbers (“Ref. #”) for each locality. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Locality F_{ST} values											
	Nec-01	**Nec-02**	**Nec-03**	**Nec-04**	**Nec-05**	**Nec-06**	**Nec-07**	**Nec-08**	**Nec-09**	**Nec-10**	**Nec-11**
Nec-01	NA	0.0000	0.0000	0.0161	0.0000	0.0033	0.0000	0.0000	0.0000	NA	0.0000
Nec-02	0.0000	NA	0.0000	0.0509	0.0000	0.0319	0.0034	0.0174	0.0109	NA	0.0000
Nec-03	0.0000	0.0000	NA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	NA	0.0277
Nec-04	0.0000	0.0509	0.0000	NA	0.0000	0.0524	0.0508	0.0454	0.0518	NA	0.0000
Nec-05	0.0000	0.0000	0.0000	0.0000	NA	0.0000	0.0000	0.0000	0.0000	NA	0.0121
Nec-06	0.0033	0.0319	0.0000	0.0524	0.0000	NA	0.0311	0.0176	0.0264	NA	0.0000
Nec-07	0.0000	0.0034	0.0000	0.0508	0.0000	0.0311	NA	0.0196	0.0061	NA	0.0000
Nec-08	0.0000	0.0174	0.0000	0.0454	0.0000	0.0176	0.0196	NA	0.0170	NA	0.0000
Nec-09	0.0000	0.0109	0.0000	0.0518	0.0000	0.0264	0.0061	0.0170	NA	NA	0.0000
Nec-10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nec-11	0.0000	0.0000	0.0000	0.0000	0.0121	0.0000	0.0000	0.0000	0.0000	NA	NA

Appendix Table C.5 Population subdivision (F_{ST}) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the San Jacinto River basin as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes and reference numbers ("Ref. #") for each locality. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Locality F_{ST} values									
	San-1	**San-2**	**San-3**	**San-4**	**San-5**	**San-6**	**San-7**	**San-8**	**San-9**
San-1	NA	0.0814	NA	0.0800	0.0474	NA	0.1169	0.0000	0.0533
San-2	0.0814	NA	NA	0.0677	0.0966	NA	0.0921	0.0000	0.0608
San-3	NA	NA	NA	NA	NA	NA	NA	NA	NA
San-4	0.0800	0.0677	NA	NA	0.1186	NA	0.0778	0.0000	0.0297
San-5	0.0474	0.0966	NA	0.1186	NA	NA	0.1323	0.0510	0.0871
San-6	NA	NA	NA	NA	NA	NA	NA	NA	NA
San-7	0.1169	0.0921	NA	0.0778	0.1323	NA	NA	0.0167	0.0604
San-8	0.0000	0.0000	NA	0.0000	0.0510	NA	0.0167	NA	0.0000
San-9	0.0533	0.0608	NA	0.0297	0.0871	NA	0.0604	0.0000	NA

Appendix Table C.6 Population subdivision (F_{ST}) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the Sabine River basin as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes and reference numbers ("Ref. #") for each locality. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Locality F_{ST} values						
	Sab-1	**Sab-2**	**Sab-3**	**Sab-4**	**Sab-5**	**Sab-6**
Sab-1	NA	NA	NA	NA	NA	NA
Sab-2	NA	NA	0.0221	0.0293	0.0204	NA
Sab-3	NA	0.0221	NA	0.0464	0.0350	NA
Sab-4	NA	0.0293	0.0464	NA	0.0755	NA
Sab-5	NA	0.0204	0.0350	0.0755	NA	NA
Sab-6	NA	NA	NA	NA	NA	NA

Appendix Table C.7 Population subdivision (F_{ST}) of Alligator Snapping Turtles (AST; *Macrochelys temminckii*) in the Trinity River basin as identified by fastSTRUCTURE analyses. See Appendix Table C.1 for sample sizes and reference numbers ("Ref. #") for each locality. Note: population-level statistics like F_{ST} should be interpreted with caution when $n < 3$.

Locality F_{ST} values								
	Tri-1	**Tri-2**	**Tri-3**	**Tri-4**	**Tri-5**	**Tri-6**	**Tri-7**	**Tri-8**
Tri-1	NA	NA	NA	NA	NA	NA	NA	NA
Tri-2	NA	NA	0.0575	-0.0100	0.0064	0.1101	NA	0.0387
Tri-3	NA	0.0575	NA	0.0537	0.0001	0.0519	NA	0.1537
Tri-4	NA	-0.0100	0.0537	NA	0.0228	0.0958	NA	0.0393
Tri-5	NA	0.0064	0.0001	0.0228	NA	0.0843	NA	0.1461
Tri-6	NA	0.1101	0.0519	0.0958	0.0843	NA	NA	0.2385
Tri-7	NA	NA	NA	NA	NA	NA	NA	NA
Tri-8	NA	0.0387	0.1537	0.0393	0.1461	0.2385	NA	NA

Appendix C Literature Cited

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Appendix D – Morphometric Data for Compiled for the Current Study

Includes the full suite of morphometric measurements collected during the current study, submitted by key contributors, and used in analyses. Data are reported by agency, metapopulation, age class from the proposed age-size class matrix, and sex.

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
Current study	NS	Adult	F	370	398	326	113	149	273	266	99	11.3
Current study	NS	Adult	F	401	434	356	135	159	310	302	77	14.6
Current study	NS	Adult	F	382	411	339	129	141	286	283	97	12.4
Current study	NS	Adult	F	388	419	321	131	149	299	274	82	13.5
Current study	NS	Adult	M	371	400	328	117	145	284	272	132	12.6
Current study	NS	Adult	M	416	437	342	144	197	318	302	92	17.7
Current study	NS	Adult	M	517	552	457	172	190	375	389	178	27.7
Current study	NS	Adult	M	340	376	302	108	132	267	245	95	9.0
Current study	NS	Adult	M	512	545	426	165	195	393	363	170	29.3
Current study	NS	Adult	M	513	556	445	160	195	384	375	186	30.3
Current study	NS	Adult	M	419	448	341	121	163	314	282	76	15.5
Current study	NS	Adult	M	419	453	358	130	158	296	306	125	16.3
Current study	NS	Adult	M	528	563	427	178	171	381	359	210	30.4
Current study	NS	Adult	M	475	509	414	156	180	325	342	162	24.6
Current study	NS	Juvenile	U	94	104	89	31	41	71	70	22	0.3
Current study	NS	Juvenile	U	230	247	202	74	87	173	166	57	2.8
Current study	RCS	Adult	F	398	428	354	129	174	320	312	95	17.7
Current study	RCS	Adult	F	343	366	304	106	145	246	255	99	10.2
Current study	RCS	Adult	M	441	481	387	138	176	329	320	143	22.6
Current study	RCS	Juvenile	U	242	267	218	78	104	183	182	46	3.7
Current study	RCS	Juvenile	U	238	256	210	80	100	179	183	42	1.4
Current study	RCS	Sub-adult	U	295	318	278	99	113	230	237	73	6.9
Current study	RCS	Sub-adult	U	267	288	238	92	111	199	200	86	4.7
Current study	RCS	Sub-adult	U	283	306	253	96	118	221	214	54	6.0
Current study	ST	Adult	F	394	418	327	117	NR	300	284	88	13.3

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
Current study	ST	Adult	F	425	452	343	147	332	326	287	64	17.7
Current study	ST	Adult	F	343	360	295	111	NR	261	253	81	10.1
Current study	ST	Adult	F	498	536	406	147	207	384	349	111	29.4
Current study	ST	Adult	F	348	372	302	103	147	265	254	76	10.4
Current study	ST	Adult	F	333	357	288	101	132	249	246	77	8.3
Current study	ST	Adult	F	477	490	389	147	164	373	320	73	23.5
Current study	ST	Adult	F	327	357	288	100	133	248	238	78	8.4
Current study	ST	Adult	F	395	425	316	114	146	295	278	101	14.6
Current study	ST	Adult	F	456	473	379	143	152	346	323	105	20.4
Current study	ST	Adult	F	449	473	373	133	157	344	318	93	18.2
Current study	ST	Adult	M	549	593	452	183	188	397	332	177	37.0
Current study	ST	Adult	M	368	382	300	111	NR	269	250	100	10.4
Current study	ST	Adult	M	456	498	381	141	188	345	331	117	22.4
Current study	ST	Adult	M	510	551	429	160	197	409	354	85	30.9
Current study	ST	Adult	M	594	663	520	187	229	445	444	157	49.7
Current study	ST	Adult	M	355	387	325	114	118	268	257	94	11.5
Current study	ST	Adult	M	418	452	368	125	164	304	313	131	17.8
Current study	ST	Adult	M	510	557	433	160	207	398	355	123	30.4
Current study	ST	Adult	M	469	504	411	138	163	355	334	182	22.3
Current study	ST	Adult	M	448	479	385	137	165	338	326	160	21.0
Current study	ST	Adult	M	627	697	524	204	230	437	435	245	56.8
Current study	ST	Adult	M	529	561	432	164	162	378	431	164	30.4
Current study	ST	Adult	M	470	516	405	143	181	354	334	133	24.0
Current study	ST	Adult	M	542	588	468	167	198	400	357	195	36.6
Current study	ST	Adult	M	491	551	421	155	177	372	352	145	22.7
Current study	ST	Adult	M	513	549	432	157	174	358	358	204	28.7
Current study	ST	Adult	U	405	433	337	120	147	298	285	105	14.1
Current study	ST	Adult	U	348	371	311	109	147	261	250	90	10.6
Current study	ST	Adult	U	333	358	272	101	132	251	233	99	8.5
Current study	ST	Adult	U	343	370	312	106	133	264	270	74	9.1

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
Current study	ST	Adult	U	342	370	289	98	149	252	250	85	7.9
Current study	ST	Adult	U	434	475	376	129	182	321	313	134	20.1
Current study	ST	Adult	U	357	388	297	111	118	262	256	68	10.2
Current study	ST	Juvenile	U	235	260	194	75	94	174	155	70	3.3
Current study	ST	Juvenile	U	222	241	189	78	86	164	152	51	2.9
Current study	ST	Juvenile	U	143	154	117	45	50	106	99	32	0.6
Current study	ST	Juvenile	U	218	231	186	68	82	160	160	59	2.3
Current study	ST	Juvenile	U	228	248	201	74	89	170	166	47	2.9
Current study	ST	Juvenile	U	209	226	183	78	81	157	145	53	2.0
Current study	ST	Sub-adult	U	292	313	252	92	NR	212	210	61	8.7
Current study	ST	Sub-adult	U	316	340	275	98	121	237	234	69	7.3
Current study	ST	Sub-adult	U	283	305	245	92	107	209	202	72	5.8
Current study	ST	Sub-adult	U	274	295	226	87	264	205	189	74	5.2
Current study	ST	Sub-adult	U	287	310	248	89	113	228	207	71	5.5
TAMUC	RCS	Adult	F	380	NR	314	NR	NR	312	282	51	14.5
TAMUC	RCS	Adult	M	534	NR	410	NR	NR	386	367	204	38.1
TAMUC	RCS	Adult	M	365	NR	313	NR	NR	292	276	86	13.6
TAMUC	RCS	Adult	M	370	NR	324	NR	NR	293	282	94	15.4
TAMUC	RCS	Adult	U	385	NR	320	NR	NR	315	290	70	14.5
TAMUC	RCS	Juvenile	U	224	NR	203	NR	NR	175	188	50	4.1
TAMUC	RCS	Juvenile	U	212	NR	184	NR	NR	157	148	52	2.2
TAMUC	RCS	Sub-adult	U	262	NR	224	NR	NR	200	200	50	NR
TSA	ST	Adult	F	484	515	403	147	174	357	342	114	24.3
TSA	ST	Adult	F	328	357	287	104	141	261	237	71	8.3
TSA	ST	Adult	F	379	407	332	120	140	300	282	94	10.6
TSA	ST	Adult	F	488	515	242	154	184	388	NR	88	NR
TSA	ST	Adult	F	411	440	324	120	156	290	NR	77	15.5
TSA	ST	Adult	F	368	402	300	110	129	268	NR	55	11.2
TSA	ST	Adult	F	407	417	346	138	154	310	NR	70	15.8
TSA	ST	Adult	F	413	428	338	126	165	318	NR	91	17.3

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
TSA	ST	Adult	F	421	454	336	127	150	324	NR	71	16.6
TSA	ST	Adult	F	435	393	343	128	139	328	NR	80	16.4
TSA	ST	Adult	F	378	399	300	113	123	267	NR	71	7.6
TSA	ST	Adult	F	368	400	320	109	137	283	NR	90	8.4
TSA	ST	Adult	F	408	438	332	126	144	309	NR	72	16.2
TSA	ST	Adult	F	382	426	331	120	158	289	NR	97	13.4
TSA	ST	Adult	F	447	462	364	144	149	357	NR	98	16.9
TSA	ST	Adult	F	389	410	312	91	149	309	NR	64	13.9
TSA	ST	Adult	F	384	408	283	120	134	283	NR	66	12.4
TSA	ST	Adult	F	379	409	316	123	159	289	NR	86	12.8
TSA	ST	Adult	F	427	461	352	135	161	327	NR	75	17.5
TSA	ST	Adult	F	330	349	277	101	137	244	NR	89	8.6
TSA	ST	Adult	M	513	562	436	159	217	394	366	145	28.6
TSA	ST	Adult	M	560	614	477	184	217	384	382	217	43.8
TSA	ST	Adult	M	610	683	498	200	212	435	418	234	55.3
TSA	ST	Adult	M	634	672	505	200	209	464	140	240	56.3
TSA	ST	Adult	M	380	440	335	121	149	304	269	94	10.8
TSA	ST	Adult	M	365	398	300	109	148	282	247	91	8.0
TSA	ST	Adult	M	510	557	436	151	396	389	361	181	27.8
TSA	ST	Adult	M	407	426	348	124	156	313	278	118	12.6
TSA	ST	Adult	M	557	572	470	166	222	406	NR	191	NR
TSA	ST	Adult	M	396	422	334	128	152	307	NR	109	13.8
TSA	ST	Adult	M	391	424	335	122	144	309	NR	105	13.4
TSA	ST	Adult	M	369	392	302	117	125	268	NR	77	11.0
TSA	ST	Adult	M	415	392	334	123	137	285	NR	128	13.4
TSA	ST	Adult	M	457	481	364	135	158	323	NR	108	19.5
TSA	ST	Adult	M	527	563	426	188	220	386	NR	186	34.5
TSA	ST	Adult	M	489	527	403	163	187	373	NR	169	27.2
TSA	ST	Adult	M	498	537	396	155	163	355	NR	171	28.6
TSA	ST	Adult	M	414	438	347	123	147	311	NR	130	16.6

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
TSA	ST	Adult	M	501	528	391	146	175	338	NR	150	25.2
TSA	ST	Juvenile	U	233	247	190	70	104	179	NR	35	2.1
TSA	ST	Sub-adult	M	318	337	262	95	119	238	NR	65	7.3
TSA	ST	Sub-adult	M	308	332	268	96	121	239	NR	63	7.2
TSA	ST	Sub-adult	U	319	342	271	105	126	238	230	86	7.9
TSA	ST	Sub-adult	U	296	325	259	99	123	124	204	75	5.0
TT	NS	Adult	F	446	477	389	140	167	331	339	120	22.5
TT	NS	Adult	F	444	475	397	144	178	368	340	90	22.3
TT	NS	Adult	F	362	380	322	114	140	272	283	75	12.8
TT	NS	Adult	F	407	430	345	118	157	300	288	98	12.4
TT	NS	Adult	F	431	459	368	131	173	330	299	111	17.5
TT	NS	Adult	F	353	380	306	109	142	266	247	112	9.1
TT	NS	Adult	F	462	492	391	140	179	346	35	75	22.0
TT	NS	Adult	M	347	372	303	108	137	253	254	88	10.1
TT	NS	Adult	M	482	511	407	153	179	355	352	178	27.3
TT	NS	Adult	M	539	575	471	179	188	387	390	179	35.0
TT	NS	Adult	M	477	520	416	144	162	349	352	151	24.8
TT	NS	Adult	M	613	652	502	203	396	438	426	252	50.1
TT	NS	Adult	U	337	364	296	111	127	252	257	89	10.1
TT	NS	Adult	U	351	418	337	123	143	311	297	111	15.5
TT	NS	Hatchling	U	43	50	44	16	23	32	32	11	0.0
TT	NS	Hatchling	U	42	42	36	NR	23	28	29	NR	0.2
TT	NS	Hatchling	U	44	44	35	NR	22	28	28	NR	0.3
TT	NS	Hatchling	U	43	43	36	NR	23	28	28	NR	0.2
TT	NS	Hatchling	U	42	42	33	NR	27	27	27	NR	0.2
TT	NS	Hatchling	U	43	43	37	NR	22	29	29	NR	0.2
TT	NS	Hatchling	U	43	43	36	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	43	43	35	NR	22	29	29	NR	0.2
TT	NS	Hatchling	U	44	44	36	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	39	39	32	NR	22	27	27	NR	0.2

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
TT	NS	Hatchling	U	39	39	32	NR	22	26	26	NR	0.2
TT	NS	Hatchling	U	41	41	33	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	43	43	36	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	43	43	34	NR	23	28	28	NR	0.2
TT	NS	Hatchling	U	43	43	35	NR	22	30	26	NR	0.2
TT	NS	Hatchling	U	41	41	34	NR	22	27	27	NR	0.2
TT	NS	Hatchling	U	42	42	35	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	40	40	34	NR	21	27	27	NR	0.2
TT	NS	Hatchling	U	42	42	35	NR	23	28	28	NR	0.2
TT	NS	Hatchling	U	42	42	36	NR	22	28	28	NR	0.2
TT	NS	Hatchling	U	40	40	35	NR	22	27	27	NR	0.2
TT	NS	Hatchling	U	44	44	36	NR	24	29	28	NR	0.2
TT	NS	Hatchling	U	45	45	36	NR	24	28	27	NR	0.2
TT	NS	Hatchling	U	43	43	35	NR	24	28	28	NR	0.2
TT	NS	Hatchling	U	44	44	36	NR	24	29	29	NR	0.2
TT	NS	Hatchling	U	43	43	35	NR	22	25	29	NR	0.2
TT	NS	Hatchling	U	42	42	36	NR	24	29	29	NR	0.2
TT	NS	Hatchling	U	42	42	35	NR	22	27	27	NR	0.2
TT	NS	Hatchling	U	40	40	35	NR	24	28	28	NR	0.2
TT	NS	Juvenile	U	154	167	142	52	66	117	114	33	0.4
TT	NS	Juvenile	U	165	181	149	58	68	128	126	35	1.1
TT	NS	Juvenile	U	241	261	191	77	90	179	170	70	4.2
TT	NS	Juvenile	U	198	218	184	69	83	154	157	40	1.9
TT	NS	Juvenile	U	111	119	103	38	46	85	86	19	0.3
TT	NS	Juvenile	U	205	223	185	67	85	157	160	39	2.0
TT	NS	Juvenile	U	229	248	203	77	81	172	175	58	2.9
TT	NS	Juvenile	U	206	222	184	67	82	157	157	51	2.0
TT	NS	Juvenile	U	186	202	168	62	73	144	142	45	1.4
TT	NS	Juvenile	U	134	145	121	40	55	97	101	25	0.5
TT	NS	Juvenile	U	109	119	101	36	44	78	81	27	0.3

Appendix Table D.1 Full set of morphometric data compiled for Alligator Snapping Turtles (AST; *Macrochelys temminckii*). Data were collected during field efforts (Current study) and provided by researchers from Texas A&M University-Commerce (TAMUC), the Turtle Survival Alliance (TSA), and Texas Turtles (TT). Includes associated metapopulation (Meta; NS = Neches+Sabine river basins, RCS = Red+Cypress+Sulphur river basins, ST = San Jacinto+Trinity river basins), age class (A-C) based on the proposed age-size class matrix in the current study, sex (M = male, F = female, U = unknown), midline straight carapace length (mid-SCL), maximum straight carapace length (max-SCL), maximum carapace width (max-CW), head width (HW), plastron length (PL), outer plastron width (outer-PL), pre-cloacal tail length (pre-C), and mass. All body measurements are reported in millimeters (mm) and mass reported in kilograms (kg).

Agency	Meta	A-C	Sex	mid-SCL	max-SCL	max-CW	HW	mid-SD	PL	outer-PW	pre-C	Mass
TT	NS	Juvenile	U	122	133	106	41	55	92	88	25	0.4
TT	NS	Juvenile	U	143	158	129	47	65	108	108	24	0.7
TT	NS	Juvenile	U	104	116	96	34	48	80	79	24	0.3
TT	NS	Juvenile	U	91	101	85	29	42	71	69	20	0.2
TT	NS	Juvenile	U	123	136	128	41	58	96	97	24	0.5
TT	NS	Juvenile	U	46	46	37	NR	22	28	28	NR	0.2
TT	NS	Sub-adult	M	316	338	268	108	117	243	223	84	7.2
TT	NS	Sub-adult	U	291	314	247	95	107	216	214	80	5.5
TT	NS	Sub-adult	U	310	323	267	98	112	233	222	70	5.7
TT	NS	Sub-adult	U	280	302	249	92	112	214	211	65	5.5
TT	ST	Adult	F	370	385	332	121	159	293	NR	64	13.1
TT	ST	Adult	F	506	541	405	155	161	373	NR	64	32.2
TT	ST	Adult	F	495	508	399	163	209	375	NR	NR	27.6
TT	ST	Adult	F	520	526	430	165	189	383	355	70	31.8
TT	ST	Adult	M	589	631	497	189	250	430	NR	228	45.0
TT	ST	Adult	M	626	684	544	214	NR	450	NR	212	55.9
TT	ST	Adult	M	445	460	398	151	NR	336	NR	90	19.5
TT	ST	Adult	M	498	521	440	155	NR	366	NR	82	27.6
TT	ST	Adult	M	518	559	456	176	NR	355	NR	168	31.9
TT	ST	Adult	U	338	310	269	95	117	233	217	51	7.8
TT	ST	Juvenile	U	122	125	105	37	46	87	NR	108	0.4
TT	ST	None	F	NR	359	332	128	167	308	NR	98	14.8
TT	ST	Sub-adult	F	313	323	281	105	126	246	241	83	8.3
TT	ST	Sub-adult	F	294	311	247	91	115	210	207	59	4.9
TT	ST	Sub-adult	U	254	270	NR	84	NR	194	NR	NR	3.9

Appendix E – List of Species Encountered During Trapping Surveys

Data for assemblages of vertebrate species included in Appendix B were assessed for correlations at sites where Alligator Snapping Turtle (AST; *Macrochelys temminckii*) were detected versus those where AST were not detected.

Appendix Table E.1 List of taxonomic groups and counts (*n*) for all catch in the current survey. Overall relative abundance (Rel. Abund.), total number of individuals observed (N), and number 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; reptiles), Page et al. (2013; fishes), and ITIS (2021; crustaceans and mammals).

Major group	Taxonomic level	Scientific name	Common name	*n*	Rel. abund.
Crocodilian	Species	*Alligator mississippiensis*	American Alligator	10	0.011
Crustacean	Species	*Callinectes sapidus*	Blue Crab	2	0.002
Fish	Species	*Pomoxis annularis*	White Crappie	80	0.090
Fish	Species	*Atractosteus spatula*	Alligator Gar	42	0.047
Fish	Species	*Lepomis macrochirus*	Bluegill	21	0.024
Fish	Species	*Pomoxis nigromaculatus*	Black Crappie	20	0.023
Fish	Species	*Micropterus salmoides*	Largemouth Bass	18	0.020
Fish	Species	*Lepisosteus oculatus*	Spotted Gar	17	0.019
Fish	Species	*Amia calva*	Bowfin	8	0.009
Fish	Species	*Ameiurus natalis*	Yellow Bullhead	8	0.009
Fish	Species	*Ictiobus bubalus*	Smallmouth Buffalo	6	0.007
Fish	Species	*Cyprinus carpio*	Common Carp	5	0.006
Fish	Species	*Pylodictis olivaris*	Flathead Catfish	5	0.006
Fish	Family	Lepisosteidae	Unknown Gar	3	0.003
Fish	Class	Teleostei	Unknown Fish	3	0.003
Fish	Species	*Ictalurus furcatus*	Blue Catfish	2	0.002
Fish	Species	*Lepomis microlophus*	Redear Sunfish	2	0.002
Fish	Species	*Carpiodes carpio*	River Carpsucker	2	0.002
Fish	Species	*Morone chrysops*	White Bass	2	0.002
Fish	Genus	*Pomoxis* sp.	Unknown Crappie	2	0.002
Fish	Genus	*Lepomis*	Unknown Sunfish	2	0.002
Fish	Species	*Oreochromis aureus*	Blue Tilapia	1	0.001
Fish	Species	*Dorosoma cepedianum*	Gizzard Shad	1	0.001
Fish	Species	*Lepomis humilis*	Orangespotted Sunfish	1	0.001
Fish	Species	*Lepomis miniatus*	Red Spotted Sunfish	1	0.001
Fish	Species	*Lepomis gulosus*	Warmouth	1	0.001
Fish	Family	Ictaluridae	Unknown Catfish	1	0.001
Turtle	Subspecies	*Trachemys scripta elegans*	Red-eared Slider	268	0.302
Turtle	Species	*Apalone spinifera*	Spiny Softshell	132	0.149
Turtle	Species	*Macrochelys temminckii*	Alligator Snapping Turtle	81	0.091
Turtle	Species	*Sternotherus carinatus*	Razor-backed Musk Turtle	45	0.051
Turtle	Species	*Pseudemys concinna*	River Cooter	29	0.033
Turtle	Species	*Graptemys sabinensis*	Sabine Map Turtle	9	0.010
Turtle	Species	*Chelydra serpentina*	Snapping Turtle	2	0.002
Turtle	Species	*Pseudemys texana*	Texas Cooter	1	0.001
Turtle	Species	*Apalone mutica*	Smooth Softshell	1	0.001
Turtle	Species	*Sternotherus odoratus*	Eastern Musk Turtle	1	0.001
Turtle	Family	Kinosternidae	Unknown Musk/Mud Turtle	1	0.001
Mammal	Species	*Procyon lotor*	Raccoon	1	0.001
Mollusk	Class	Bivalvia	Unknown Bivalve	1	0.001
			Total (*N*)	887	
			Number of groups (S)	47	

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Appendix F – Updated county occupancy year of record

Includes an updated county year-of-record list from USFWS (2021) and Rosenbaum et al. (2022) for known AST occupancy in east Texas based on results from the current study. Also includes sources for recent studies confirming occupancy in applicable counties.

Appendix Table F.1 Updated year of known Alligator Snapping Turtle (AST; *Macrochelys temminckii*) occupancy dates for Texas counties. Original occupancy dates based on Appendix Table D1 of the Species Status Assessment (SSA) (USFWS 2021). "NA" = county was not originally included in Appendix Table D1 of the SSA.

County	SSA Appendix Table D1	Updated year of record	Source for updated year of record
Anderson	2014	2020	Rosenbaum et al. (2022)
Angelina	2016	2021	Rosenbaum et al. (2022)
Bowie	2010	2022	Current study
Brazoria	NA	2022	Rosenbaum et al. (2022)
Camp	Unknown	2021	Rosenbaum et al. (2022)
Cass	2014	2021	Rosenbaum et al. (2022)
Chambers	Unknown	2022	Current Study
Cherokee	2013	2022	Current Study
Collin	2002	2020	Rosenbaum et al. (2022)
Dallas	Unknown	2021	Franklin et al. (2021)
Delta	Unknown	2021	Rosenbaum et al. (2022)
Fannin	1993	-	
Franklin	1986	-	
Freestone	2013	-	
Galveston	NA	2021	Norrid et al. (2021)
Grayson	1993	-	
Gregg	2013	-	
Hardin	2018	2022	Current Study
Harris	2019	2022	Current Study; Munscher et al. (2023)
Harrison	2015	2021	Rosenbaum et al. (2022)
Henderson	2014	-	
Hopkins	2013	-	
Houston	1986	2020	Rosenbaum et al. (2022)
Hunt	NA	2021	Current Study
Jasper	2016	2021	Current Study
Jefferson	2013	2022	Current Study
Kaufman	NA	2021	Rosenbaum et al. (2022)
Lamar	1993	2022	Current Study; Hughes et al. (2023)
Leon	2013	2020	Rosenbaum et al. (2022)
Liberty	2016	2022	Current Study
Madison	2017	-	
Marion	2009	2022	Current Study
Montgomery	2019	-	
Morris	Unknown	-	
Nacogdoches	2001	2021	Rosenbaum et al. (2022)
Navarro	NA	2021	Rosenbaum et al. (2022)
Newton	2000	2021	Current Study
Orange	2013	2021	Current Study
Panola	2004	-	
Polk	2013	2022	Current Study
Rains	1985	2021	
Red River	2013	2022	Current Study
Rockwall	Unknown	-	

Appendix Table F.1 Updated year of known Alligator Snapping Turtle (AST; *Macrochelys temminckii*) occupancy dates for Texas counties. Original occupancy dates based on Appendix Table D1 of the Species Status Assessment (SSA) (USFWS 2021). "NA" = county was not originally included in Appendix Table D1 of the SSA.

County	SSA Appendix Table D1	Updated year of record	Source for updated year of record
Rusk	2016	-	
Sabine	2000	-	
San Augustine	Unknown	2020	Rosenbaum et al. (2022)
San Jacinto	2000	2022	Current Study
Shelby	2016	2021	Rosenbaum et al. (2022)
Smith	2014	-	
Tarrant	2018	2022	Current Study
Titus	2013	2020	Rosenbaum et al. (2022)
Trinity	Unknown	2021	Rosenbaum et al. (2022)
Tyler	2010	2020	Rosenbaum et al. (2022)
Upshur	Unknown	2021	Rosenbaum et al. (2022)
Van Zandt	Unknown	2021	Rosenbaum et al. (2022)
Walker	2000	2020	Rosenbaum et al. (2022)
Waller	NA	2021	Rosenbaum et al. (2022)
Wood	2001	2020	Rosenbaum et al. (2022)

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