Alligator Snapping Turtle (*Macrochelys temminckii*) Occupancy Increases in Higher-Order Streams

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Abstract - *Macrochelys temminckii* (Alligator Snapping Turtle) is a large freshwater turtle that occurs in a broad range of stream orders in the southeastern US. The species has experienced range contraction and population declines throughout its range. Environmental DNA (eDNA) sampling is an effective method for indirect detection of the Alligator Snapping Turtle when trapping or radio tracking is not feasible. Environmental conditions and resource availability vary across stream orders, potentially influencing Alligator Snapping Turtle occupancy patterns and eDNA detection probabilities. In this study, we sampled eDNA to survey for the Alligator Snapping Turtle in the Sam Houston National Forest, TX, to determine if Alligator Snapping Turtle occupancy and detectability were correlated with stream order and its associated abiotic variables. We found that occupancy was indistinguishable among stream orders 1–4, but that occupancy was significantly higher in fifth-order streams, potentially due to more stable water temperature and greater resource availability. Furthermore, our models suggest that no abiotic variables significantly affected detection probability. This information provides a better understanding of how the Alligator Snapping Turtle may be distributed across stream orders and may help inform forest-management practices near streams where the species may occur.

Introduction

Macrochelys temminckii (Troost in Harlan) (Alligator Snapping Turtle) is a large-bodied, long-lived species of freshwater turtle widely distributed across the southeastern US that has experienced range contraction and population declines, especially at the periphery of its range (Carr et al. 2023a, Garig et al. 2021, Kessler et al. 2017, Pritchard 2006, Riedle et al. 2008a, Rosenbaum et al. 2023a, TTWG 2025). As such, the Alligator Snapping Turtle is considered a species of greatest conservation need in many states across its range and has been proposed as threatened under the US Endangered Species Act (Christensen et al. 2024, USFWS 2021). The Alligator Snapping Turtle is highly aquatic and highly benthic in its habits (Carr et al. 2023b, Pritchard 2006), with females rarely leaving the water except for nesting (Carr et al. 2023b, Ernst and Lovich 2009, Micek et al. 2024). Because of its highly aquatic nature, this species is rarely encountered in the wild, which complicates monitoring and surveying efforts. The use of trapping and radio telemetry (e.g., Adams et al. 2024, Cozad et al. 2023, Fitzgerald and Nelson

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2011, Riedle et al. 2008b) has helped to document habitat use and occupancy of the Alligator Snapping Turtle. However, these methods tend to be highly time- and resource-intensive, with the potential to cause stress, injury, or death to animals (Cash et al. 1997, Larocque et al. 2012, Schalk et al. 2025).

Sampling for environmental DNA (eDNA), the genetic material naturally shed by organisms into their environment, serves as a non-invasive alternative to conventional methods of determining species occurrence in aquatic systems (e.g., trapping; Ficetola et al. 2008). Since its development, eDNA sampling has become a widely used tool for indirectly determining the presence or absence of a species in an area, and it is particularly useful for rare and cryptic species (Ficetola et al. 2008, Goldberg et al. 2015, Rees et al. 2014, Thomsen et al. 2011). However, eDNA detection can be influenced by both intrinsic and extrinsic factors such as the rate of eDNA shedding by a species (Klymus et al. 2015) and how long eDNA persists in the environment (Sassoubre et al. 2016). Non-avian reptiles, for example, may shed eDNA more slowly than fishes or amphibians due to a keratinized rather than mucosal exterior integument (Adams et al. 2019). However, ectotherms may experience increased rates of eDNA shedding with higher metabolic rates and increased activity at higher temperatures (Brown et al. 2004, Yang et al. 2025), while lower water temperatures have been shown to slow the rate of eDNA shedding in ectotherms (Eichmiller et al. 2016). Higher temperatures may promote increased microbial activity, causing eDNA to degrade more quickly (Pilliod et al. 2013, Strickler et al. 2015). Likewise, fast-flowing water may dilute eDNA more quickly, but it may also keep it suspended in the water column, where it can be collected via a sample of surface water (Curtis et al. 2021, Jane et al. 2015, Woytek 2025).

Across different life stages, the Alligator Snapping Turtle generally selects habitats with a high percentage of canopy cover and structural features such as stream banks or submerged woody debris (Howey and Dinkelacker 2009, Moore et al. 2014, Spangler et al. 2021) and has been documented in both shallow-water (Harrel et al. 1996, Huntzinger et al. 2019) and deep-water (Howey and Dinkelacker 2009, Lescher et al. 2013) habitats. Preference for water depth may change based on time of year, with turtles moving to deeper water in the winter and shallower water in the early summer (Riedle et al. 2006). Ontogenetic shifts in habitat selection have been documented, with smaller, younger turtles preferring smaller streams to avoid predation, while adult turtles may inhabit larger streams where more food and space is available (Riedle et al. 2006, Spangler et al. 2021). However, in the Pascagoula River Drainage of southern Mississippi and Alabama, juvenile Alligator Snapping Turtles were found across a wide span of stream sizes, with stream widths varying from 6.4–140 m (Brown 2023).

Understanding the habitat use and spatial distribution of at-risk species, such as the Alligator Snapping Turtle, is crucial in the context of land management. If we can predict stream-order occupancy by Alligator Snapping Turtles, directed efforts can be made for implementation of management practices and guidelines that minimize water-quality changes resulting from proximal habitat modifications including timber harvesting, channelization, and impoundment (Bodie 2001, Lickwar et al. 1992, Sterrett et al. 2011). Lower-order streams are typically more abundant and dynamic on landscapes as compared to higher-order streams (Botter et al. 2026). Given their larger spatial extent, it is important to understand the occurrence of Alligator Snapping Turtles in lower-order streams relative to higher-order streams, especially in the context of project planning (e.g., timber harvest) on multi-use landscapes. In these scenarios, management activities may require mitigation measures such as modifying forest roads and timber skid trails to minimize soil rutting (Cristan and Potter 2024), or maintaining vegetative buffers along streams to reduce inflow of sediments (Bodie 2001).

In this study, we sampled 45 sites spanning 5 stream orders across Sam Houston National Forest (SHNF) in eastern Texas to investigate patterns of occupancy and detection for the Alligator Snapping Turtle. We hypothesized that occupancy would be positively associated with increasing stream order since higher-order streams are larger and generally contain greater diversity of microhabitats. We also predicted that detection probability would be higher in cooler waters since lower temperatures tend to preserve eDNA better than high temperatures. Likewise, we predicted that higher stream-current velocity would be associated with higher detection probability since high-velocity currents tend to suspend eDNA for longer periods than low-velocity currents. Lastly, we predicted higher detection probability in waters with lower turbidity, as increased particulates in water may interfere with eDNA amplification (McKee et al. 2015). By understanding the patterns of occupancy and detection probability of the Alligator Snapping Turtle in relation to stream order and abiotic variables, we can make inferences on their distribution across the landscape to guide managers in facilitating project planning or developing mitigation measures.

Field-site Description

The SHNF is one of the national forests in Texas which were established by an act of the Texas legislature in 1933 that authorized the purchase of lands for the national forest system. The SHNF is located ~80 km north of Houston, TX, within the West Gulf Coastal Plain Pineywoods ecoregion. The forest spans 65,978 ha across Montgomery, Walker, and San Jacinto counties. The upland forest cover is characterized as *Pinus* (pine) and mixed pine–hardwood stands, with the bottomland forest being mainly mixed hardwood stands (Rudolph and Conner 1991). *Pinus echinata* Miller (Shortleaf Pine) and *P. taeda* L. (Loblolly Pine) are the dominant forest cover in the Pineywoods ecoregion. Streams in the SHNF are within the San Jacinto watershed and drain into the West Fork of the San Jacinto River (Rosenbaum et al. 2023a). Past surveys have documented the Alligator Snapping Turtle in the San Jacinto watershed, and this watershed along with the Trinity watershed likely forms the western boundary of the species' distribution in Texas (Rosenbaum et al. 2023a, b).

Methods

We conducted this study within the SHNF in Walker County, TX. Using the United States Geological Survey's National Hydrography Dataset (USGS 2024), we located sites that included from first- to fifth-order streams (Fig. 1). While we initially planned to survey 10 sites per stream order (for a total of 50 sites), only 7 and 8 sites were accessible for fourth- and fifth-order streams, respectively (n = 45 sites total). Beyond consideration of stream order, we selected sites based on their accessibility and proximity to a road. At each survey site, we collected 3 samples spaced ~10 m apart. We conducted all sampling in March 2024, as first- and second-order streams in this region can dry by late spring or early summer.

To ensure we had no eDNA contamination, we used sterile plastic bottles (Nalgene®; Rochester, NY) to collect three 1.0-L water samples prior to measuring physical and chemical water parameters (water turbidity, temperature, pH, salinity, conductivity, total dissolved solids [TDS], dissolved oxygen [DO], and flow velocity) at each site. We used a multi-parameter meter (APERA® Instruments PC60; Columbus, OH) to measure water temperature, pH, salinity, conductivity, and TDS. We measured DO with an APERA® Instruments SX716 DO meter and turbidity with an APERA® Instruments TN400 portable turbidity meter. We measured both surface and benthic current velocities of the stream channel with

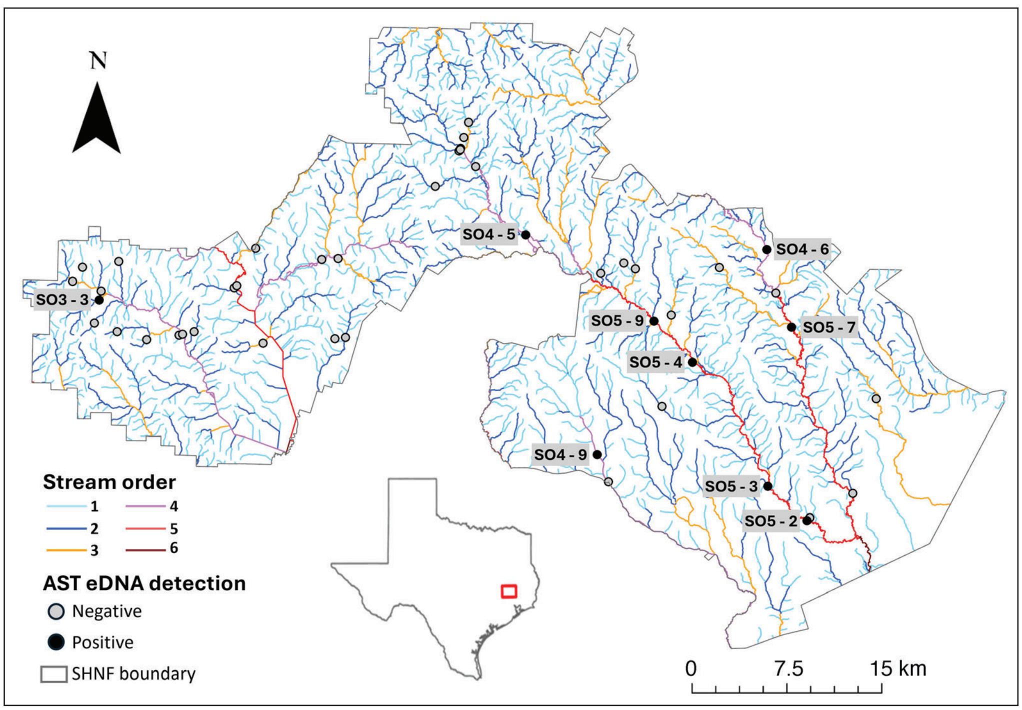

Figure 1. The stream network in Sam Houston National Forest, TX, with streams color-coded by stream order (SO). Points represent the 45 sites that were sampled for *Macrochelys temminckii* (Alligator Snapping Turtle) eDNA in March 2024. The black points labeled with SO number followed by their site code indicate sites with positive eDNA detection. Light gray points indicate sites with negative eDNA detection.

a Swoffer® Instruments Model 2100 flow meter (Sumner, WA). We placed water samples on ice within 30 minutes of collection for transport to the laboratory, and samples remained on ice until filtering. Additionally, we thoroughly rinsed all sampling equipment with distilled water and cleaned with sterile Kimtech Kimwipes® (Kimberly-Clark, Irving, TX) to remove and ensure no residual eDNA on sampling equipment between sites.

We filtered water samples at 500–600 mm Hg vacuum pressure within 48 hours of collection using a Rocker Scientifc 400® vacuum pump (Kaohsiung City, Taiwan). Each 1.0-L water sample was filtered with two 250-mL filter funnels (Thermo Scientific Nalgene®; Walthan, MA) each containing a 0.45-µm pore filter. Filtering continued until the entire 1.0-L sample was filtered or a filtering time of 240 min was reached. We then removed each of the 2 filters from the filter funnels, and folded and placed them in 2-mL microcentrifuge tubes (VWR®; Avantor, Radnor, PA) with site and date labels. We then froze the tubes at -20 °C and shipped them to Tangled Bank Conservation (Asheville, NC) for eDNA amplification. Filters were cut in half, and we used Zymo Research Quick-DNA Miniprep Plus Kit (Irvine, CA) to extract the DNA from 1 half using a modified protocol (Spear et al. 2015). We saved and stored the unextracted filter halves at -80 °C. We performed presence/absence qPCR (Quant Studio 3; Thermo Fisher Scientific) assays using a set of 2 primers designed by Kessler et al. (2020) to amplify a 110-bp section of the mtDNA control region of the Alligator Snapping Turtle genome (Table 1). The eDNA samples in this study were run in a 20-µL reaction volume consisting of 10 µL of PerfeCTa qPCR ToughMix L-ROX (Quantabio, Beverly, MA), 1 µL of each primer at 10 µM and probe at 5 µM, 3.5 µL nuclease free water, and 3.5 µL of eDNA sample extract, or 1µL positive control sample with 2.5 µL of water. We followed a qPCR thermocycler protocol as follows: 15 min at 95 °C, 50 cycles of 94 °C for 60 sec, and an annealing temperature of 60 °C, with data collection during the annealing stage. We first ran samples with an internal positive control (TaqMan Exogenous Internal Positive Control Reagents; Thermo Fisher Scientific) according to the associated protocol. We treated inhibited samples with OneStep PCR Inhibitor Removal Kits (Zymo Research). For each sample, we ran the primer set in triplicate with 1 negative control. We considered samples positive when the negative control failed to amplify, and at least 2 of the 3 runs tested positive for Alligator Snapping Turtle DNA. There were no cases where the negative control amplified.

Table 1. Oligonucleotide (Oligo) names and sequences of the primer set designed by Kessler et al. (2020) and used to amplify a 110-bp section of *Macrochelys temminckii* (Alligator Snapping Turtle) mitochondrial DNA with their starting size (Start) of the synthesis reaction, sequence length, melting temperature (Tm), and percent composition of guanine and cytosine bases (GC%).

Oligo	Name	Sequence	Start	Length	Tm	GC%
Forward	AST-DLP-3F	5′-CCC TTT GAC GCA GGG AAT AG−3′	47	20	62.330	55
Reverse	AST-DLP-3R	5′-GCA CGA TAT ACA TAG GGK GGT ATA G−3′	156	25	61.831	44
Probe	AST-DLP-P	5′−6-FAM TCC GGC GCT TGT CGC CAT ATT TA-TAMRA−3′	70	23	68.281	52.174

We analyzed eDNA results with respect to stream order (Strahler 1957) and all abiotic variables. We averaged surface and benthic current velocities for each site, but if the water was too shallow for 2 separate velocity measurements, we used the sole measurement as the average. We treated each sample as an independent survey, resulting in 3 surveys at each site. We then constructed a detection history based on the eDNA results at each site, where a 1 indicates that eDNA was detected and 0 indicates that eDNA was not detected. Because each site's surveys were collected in rapid succession, we measured all variables for detection at the site-level.

We used the 'unmarked' (v. 1.2.5) package (Kellner et al. 2023) in R (v. 4.4.2; R Foundation, Vienna, Austria) to format our data for analysis, and the 'ubms' (v. 1.2.8) package to fit Bayesian occupancy models (Kellner et al. 2021). We limited candidate models to 5 variables to avoid overparameterization. Given the centrality of stream order to our research question, we included stream order as a categorical variable relative to first-order streams on occupancy in all candidate models. Each candidate model then considered a single abiotic water variable on detection probability (Table 2). Additionally, we ran a model that contained stream order on occupancy but no variable on detection probability and considered this variable as a null model. Each parameter used the package's default vague priors (logistic [0,1]) and consisted of four 25,000-iteration chains. We checked each chain's Gelman–Rubin statistic to verify convergence. Finally, we compared candidate models using leave-one-out information criterion (LOOIC), which is interpreted similarly to Akaike information criterion (AIC) and Watanabe–Akaike information criterion (WAIC) but is more robust against outliers (Vehtari et al. 2017).

Results

On 5–6 and 19–20 March 2024, we collected 3 water samples at 45 sites for a total of 135 water samples across stream orders (1–5) in the SHNF (Fig. 1). Water

Table 2. Descriptions of variables included in occupancy models examining detection rates of *Macrochelys temminckii* (Alligator Snapping Turtle) eDNA across stream orders in the Sam Houston National Forest, TX.

Variable name	Description
Stream order	Hierarchical classification of stream size based on level of branching; streams ranged from 1 (smallest/unbranching streams) to 5 (medium-sized rivers) in the Sam Houston National Forest
Average flow rate	Average of the surface and bottom water flow rates (m/s)
Turbidity	Surface water turbidity (NTU)
Water temperature	Surface water temperature (°C)
Total dissolved solids	Total concentration of dissolved substances (ppm) of surface water
Salinity	Surface water salinity (ppt)
Dissolved oxygen	Dissolved oxygen saturation (%) of surface water

parameters varied slightly across stream orders, with higher-order streams having higher flow velocity and lower temperatures on average compared to lower-order streams (Table 3). Surface turbidity, pH, TDS, and DO did not show a clear trend among different stream orders (Table 3).

Thirty streams were classified as stream orders 1, 2, or 3 (n = 10 each). Seven sites were classified as stream order 4, and 8 sites were classified as stream order 5 for a total of 45 sites allowing for the collection of 135 water samples (i.e., 3 samples per site). We detected eDNA of the Alligator Snapping Turtle at 9 of the 45 sites (Fig. 1). Stream order 5 sites (n = 8) had the highest proportion of positive detections with 5 of 8 sites (62.5%) yielding at least 1 water sample testing positive for eDNA. Stream order 4 sites (n = 7) had 3 sites (42.9%) testing positive, while stream order 3 sites had only 1 positive detection site (10.0%). There were no eDNA detections in stream orders 1 and 2 (Fig. 2). In addition to the eDNA detection frequency of sites, we also report the positive eDNA detection frequency of samples among stream orders. Eight of 24 water samples (33.3%) from stream order 5 sites tested positive for Alligator Snapping Turtle eDNA (Fig. 2). Stream order 4 had 5 of 21 samples (23.8%), and stream order 3 had 2 of 30 (6.7%) positive samples, with both samples occurring within a single site (SO3 - 3; Fig 1).

The best-supported occupancy model considered stream order on occupancy (β_{SO2} = -1.43, 95% CI = -4.72–0.97; β_{SO3} = -0.31, 95% CI = -2.50–1.67; β_{SO4} = 1.90, 95% CI = -0.27–4.81; β_{SO5} = 2.32, 95% CI = 0.34–4.91) and DO on detection probability (β = -1.44, 95% CI = -3.26–0.40). Three additional models were within 2 ΔLOOIC, indicating comparable model fit. However, all detection parameter estimates contained zero within their 95% credible intervals, and therefore none were considered significant. Likewise, all models found non-significant effects of stream orders 2, 3, and 4, but a significantly positive effect of stream order 5. Collectively, all candidate models suggest that occupancy by the Alligator Snapping Turtle is

Table 3. Mean (standard error) of environmental variables measured at each site during water sample collection for *Macrochelys temminckii* (Alligator Snapping Turtle) eDNA in March 2024 at the Sam Houston National Forest, TX. Surf. turb. = surface turbidity, flow vel. = flow velocity, cond. = conductivity, temp. = temperature, and TDS = total dissolved solids.

	Stream order				
	1 (n = 10)	2 (n = 10)	3 (n = 10)	4 (n = 7)	5 (n = 8)
Surf. turb. (NTU)	23.44 (4.42)	21.75 (3.91)	14.48 (2.74)	19.05 (2.57)	18.47 (3.73)
Flow vel. (m/s)	0.039 (0.026)	0.066 (0.033)	0.066 (0.025)	0.108 (0.044)	0.149 (0.080)
Temp. (°C)	19.76 (0.65)	19.43 (0.69)	18.11 (0.86)	17.79 (1.33)	16.60 (0.70)
pH	6.88 (0.14)	6.94 (0.10)	7.05 (0.15)	6.97 (0.17)	7.04 (0.12)
Cond. (µS/cm)	269.0 (51.9)	342.9 (105.2)	374.9 (83.9)	300.9 (60.8)	301.9 (35.9)
TDS (ppm)	190.9 (36.7)	243.2 (75.0)	266.1 (59.6)	213.6 (43.1)	214.4 (25.5)
Salinity (ppt)	0.133 (0.025)	0.173 (0.053)	0.188 (0.042)	0.150 (0.030)	0.152 (0.018)
DO (%)	66.04 (8.23)	88.66 (3.90)	73.10 (9.68)	89.35 (4.90)	79.69 (2.38)

positively associated with fifth-order streams, but that detection is not associated with any physical or chemical water quality variables (Table 4).

Discussion

Our results suggest that in early spring, the Alligator Snapping Turtle has a positive association with higher-order streams and is scarcely found in first- and second-order streams. We observed that eDNA of the Alligator Snapping Turtle was only detected in stream orders 3 through 5, and frequency of detection increased with increasing stream order. Despite this pattern, fifth-order streams were the only order to yield a statistically significant relationship with Alligator Snapping Turtle occupancy. Further, although multiple physical and chemical water quality variables were included in our top models, none of those variables had a statistically significant relationship with detection probability.

Greater rates of occupancy by the Alligator Snapping Turtle in higher-order streams may be due to preferences for larger streams with more expansive

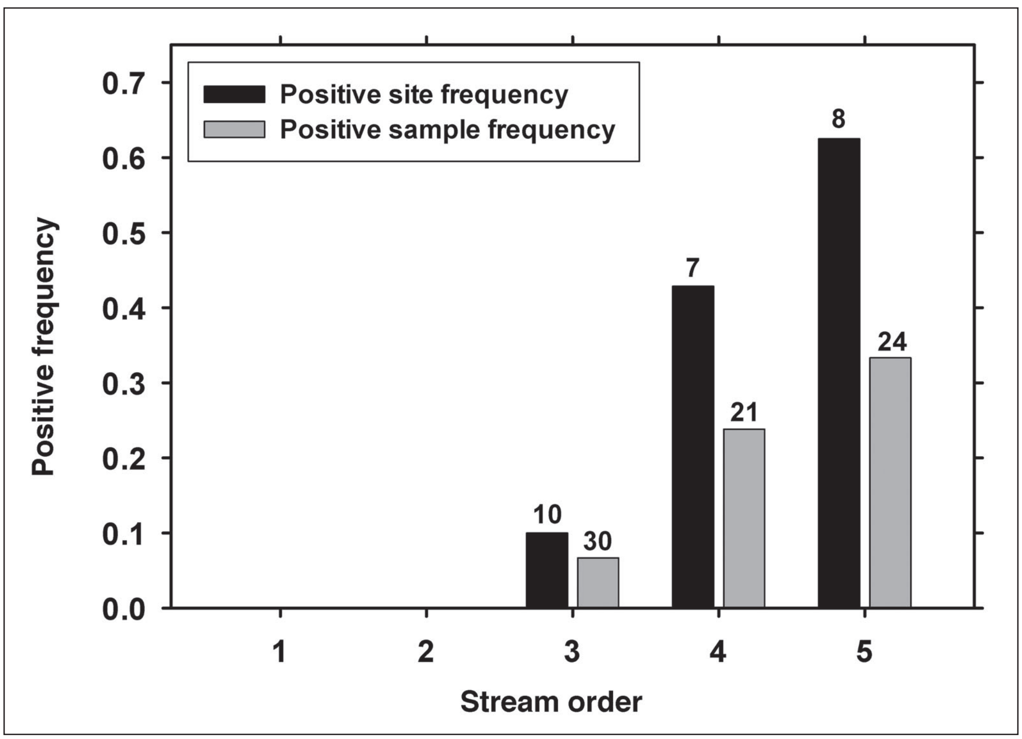

Figure 2. The frequency or proportion of positive eDNA detections for sites and samples among stream orders in the Sam Houston National Forest, TX. Sites (n = 45) were positive if at least 1 of the 3 water samples collection within each site tested positive for *Macrochelys temminckii* (Alligator Snapping Turtle) eDNA. The total number of sites belonging to each stream order (n = 10 sites for stream orders 1, 2, and 3, n = 7 sites for stream order 4, and n = 8 sites for stream order 5) are shown above each black bar and used to calculate frequency (i.e., ratio of positive to total sites). Likewise, the total number of water samples (n) is shown above each gray bar and used to calculate positive sample frequency.

floodplains (Howey and Dinkelacker 2009, Lescher et al. 2013, Pearson et al. 2023). Larger stream orders may have more submerged structure due to their greater volume of water, thus providing more resources such as food and refugia for turtles (Rosenbaum et al. 2023b). In a telemetry study of subadult Alligator

Table 4. Ranked candidate occupancy models of *Macrochelys temminckii* (Alligator Snapping Turtle) based on eDNA detections with relation to stream order (SO). Models were fitted with eDNA data from 45 sites (3 samples per site) across the Sam Houston National Forest, TX (n = 135). Models are ranked based on leave-one-out information criteria (LOOIC), which is interpreted identically to Akaike's information criterion. Statistically significant variables/levels are denoted by an asterisk (*). DO = dissolved oxygen, TDS = total dissolved solids.

Model rank (prominent variable)	LOOIC	Estimated coefficients (95% credible intervals) Ψ	Estimated coefficients (95% credible intervals) ρ
1 (DO)	0.00 (72.995)	Intercept: -1.67 (-3.18 , -0.34)* SO_2: -1.43 (-4.72, 0.97) SO_3: -0.31 (-2.50, 1.67) SO_4: 1.90 (-0.27, 4.81) SO_5: 2.32 (0.34, 4.91)*	Intercept: -0.00 (-0.91, 0.92) Oxygen: -1.43 (-3.26, 0.40)
2 (TDS)	1.05 (74.049)	Intercept: -1.58 (-3.16 , -0.05)* SO_2: -1.56 (-4.79, 0.80) SO_3: -0.36 (-2.57, 1.63) SO_4: 1.46 (-0.57, 3.87) SO_5: 2.49 (0.37, 5.36)*	Intercept: -0.37 (-1.34, 0.60) DO: 0.62 (-0.17, 1.53)
3 (Null model)	1.17 (74.166)	Intercept: -1.73 (-3.27 , -0.33)* SO_2: -1.56 (-4.78, 0.81) SO_3: -0.34 (-2.52, 1.65) SO_4: 1.47 (-0.47, 3.87) SO_5: 2.47 (0.42, 5.17)*	Intercept: -0.19 (-1.12, 0.72)
4 (pH)	1.58 (74.579)	Intercept: -1.67 (-3.23 , -0.25)* SO_2: -1.57 (-4.86, 0.80) SO_3: -0.39 (-2.60, 1.57) SO_4: 1.42 (-0.54, 3.68) SO_5: 2.42 (0.38, 5.12)*	Intercept: -0.22 (-1.17, 0.71) pH: 0.53 (-0.36, 1.45)
5 (Turbidity)	2.705 (75.700)	Intercept: -1.71 (-3.22 , -0.33)* SO_2: -1.56 (-4.86, 0.79) SO_3: -0.27 (-2.48, 1.76) SO_4: 1.45 (-0.49, 3.73) SO_5: 2.56 (0.48, 5.42)*	Intercept: -0.17 (-1.09, 0.74) Turbidity: 0.46 (-0.64, 1.66)
6 (Water temp.)	2.726 (75.721)	Intercept: -1.37 (-3.05 , -0.34)* SO_2: -1.51 (-4.83, 0.92) SO_3: -0.44 (-2.66, 1.60) SO_4: 1.50 (-0.59, 4.04) SO_5: 2.18 (0.07, 4.84)*	Intercept: -0.67 (-1.78, 0.53) Temp.: -0.65 (-1.63, 0.38)
7 (Flow velocity)	3.928 (76.923)	Intercept: -1.74 (-3.28 , -0.32)* SO_2: -1.55 (-4.80, 0.78) SO_3: -0.34 (-2.51, 1.63) SO_4: 1.47 (-0.49, 3.75) SO_5: 2.41 (0.41, 5.06)*	Intercept: -0.12 (-1.16, 0.88) Velocity: -0.09 (-0.69, 0.48)

Snapping Turtles in Louisiana, 73.8% of location fixes were associated with structure, with turtles found beneath or within submerged logs on multiple occasions (Harrel et al. 1996). Alligator Snapping Turtles may also prefer larger streams during cold weather because water temperatures are warmer and more stable in larger streams than smaller streams (Riedle et al. 2006), with similar patterns during hot summer months during which large streams remain cooler (Howey and Dinkelacker 2009). Further, the lowest-order streams may dry up entirely in the summer and freeze over in the winter, thus prohibiting their use by Alligator Snapping Turtles.

None of the water parameters had a meaningful influence on detection probability. While these variables may influence the detection of Alligator Snapping Turtle eDNA in streams, limited variance in these variables within early spring could not clearly elucidate potential influences of water properties on detection probability. Woytek and Lutterschmidt (2025) showed eDNA persistence with wide variability in temperature and pH exposure, also suggesting that detection probability may not be influenced by natural differences in stream temperature and pH. Our results indicate that the Alligator Snapping Turtle has reliably low detection probabilities (20–50%) irrespective of habitat quality, further contributing to the species' cryptic nature. Alternatively, it is possible that the limited variation in our water parameter data (Table 3) proved insufficient to explain differences in detection. Indeed, previous work in the same region has found significantly higher rates of Alligator Snapping Turtle eDNA detection in flowing waters as opposed to still waters, presumably because eDNA quickly settles out in still waters and is thus unavailable for aquatic collection (Jane et al. 2015, Woytek 2025, Xiao et al. 2025). For this study, we were restricted to the survey sites that occurred in the SHNF, which may not include the variation in stream conditions present in other areas. Increasing the spatial and temporal extent of our sampling effort to achieve greater variation in water conditions might provide stronger insight as to whether eDNA detection can be impacted by environmental variables.

Because of the ephemeral nature of first- and second-order streams on the landscape in this region, we restricted our surveys to a single timeframe (early spring) when all stream orders had water present and available to sample. We acknowledge the seasonal influences of eDNA (e.g., Buxton et al. 2017, Sander et al. 2024, Troth et al. 2021) and the patterns of eDNA detection that may differ seasonally as influenced by seasonal changes in activity patterns of the Alligator Snapping Turtle (e.g., Hyder et al. 2021, Kessler and Dreslik 2023, Munscher et al 2021). During the Alligator Snapping Turtle nesting season (April–June), for example, there may be more positive eDNA samples from smaller stream orders as female reproductive activity increases and females transverse the landscape, potentially crossing smaller streams in search of suitable nesting sites (Carr et al. 2023b, Ernst and Lovich 2009). Additional sampling across other seasons, as well as different watersheds in the species' range, may provide a more holistic understanding of the patterns of Alligator Snapping Turtle eDNA.

The Alligator Snapping Turtle has an extensive geographic range across the southeastern US (Carr et al. 2023a, TTWG 2025), with forest cover serving as one of the best predictors of its occupancy across watersheds in our study region (Rosenbaum et al. 2023b). As such, this species has a high probability of occurrence across National Forests in the southeastern US. Federal lands, such as National Forests, are managed under a multiple-use framework and therefore open to resource extraction (e.g., timber) as well as other management objectives (e.g., wildlife habitat). Based on the lack of Alligator Snapping Turtle eDNA detected in the smallest streams in this study, project planning may not need to account for Alligator Snapping Turtles in smaller streams and thus would not need to exclude the surrounding areas from their harvest zones in early spring in this region. For areas surrounding higher-order streams in which the Alligator Snapping Turtle is present, mitigation measures such as riparian buffers, may need to be implemented to minimize impact. Timber harvesting can decrease water quality of neighboring streams, but impacts can be minimized through maintenance of forest roads and skid trails as well as the establishment of streamside management zones to reduce erosion and sedimentation of streams (Cristan et al. 2016, Warrington et al. 2017). Furthermore, the land adjacent to streams may be utilized by Alligator Snapping Turtles for nesting in the spring. Female Alligator Snapping Turtles may travel up to 196 m on land, but they typically nest within 100 m of water (24 m on average; Carr et al. 2023b). Thus, these riparian areas would also need to be taken into consideration while implementing mitigation measures during the nesting season.

Environmental DNA sampling has proven to be a highly valuable method for studying the distribution of organisms without requiring direct observation or trapping. Its use can be further expanded to address the distributional status of species across stream orders and serve to protect the riparian ecosystems inhabited by these species. The results of our study show greater Alligator Snapping Turtle occupancy in higher stream orders, which can be used to inform management practices throughout the species' range. Future studies should involve stream order-based eDNA surveys of different drainage systems within the range of the Alligator Snapping Turtle, and across seasons, to examine how species occupancy and eDNA detection vary with time and location. Sampling of eDNA may be a more time- and cost-effective method to perform species assessments before planned land management projects are carried out to ensure negative impacts on wildlife are minimized.

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