Use of Environmental DNA for Detecting Cryptic Species in Wetland Habitats: A Case Study of the Western Chicken Turtle (*Deirochelys reticularia miaria*)

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TPWD SPR-0504-383

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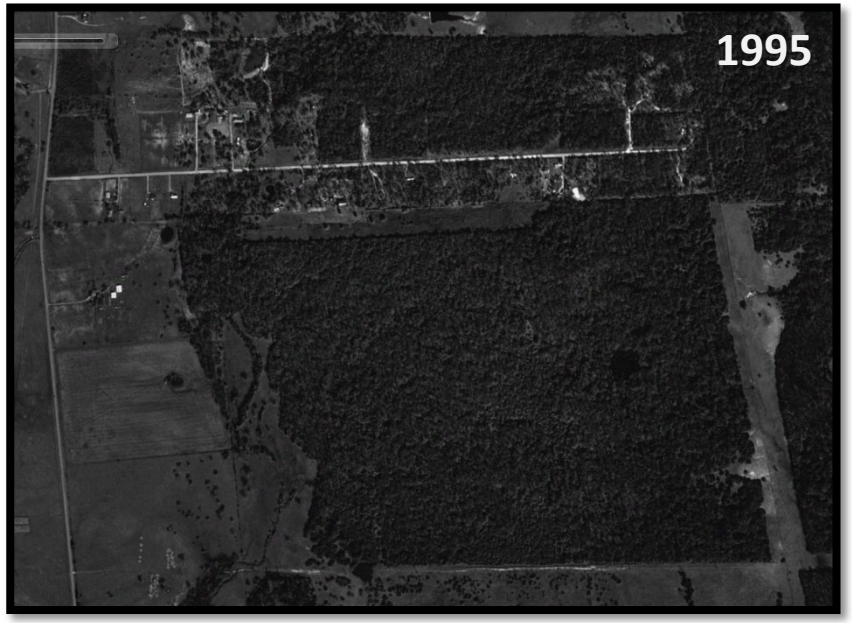
TANGLED BANK CONSERVATION™

Texas Chapter of the Wildlife Society 2023 Annual Meeting; Houston, TX
24 February 2023

Conservation Need

- Petitioned for protection under ESA[1]
- Significant 90-day findings[2]
  - 4 of 5 factors evaluated
  - SSA due in 2024
- Current status throughout range[3-8]
  - Critically imperiled – Missouri
  - Imperiled – Arkansas and Louisiana
  - Vulnerable – Mississippi
  - SGCN – Oklahoma and Texas



[1]Center for Biological Diversity 2010
[2]USFWS 2011
[3]Missouri Department of Conservation 2022
[4]Arkansas Game and Fish Commission 2005
[5]Holcomb et al. 2015
[6]Mississippi Natural Heritage Program 2018
[7]Oklahoma Department of Wildlife Conservation 2016
[8]Texas Parks and Wildlife Department 2020

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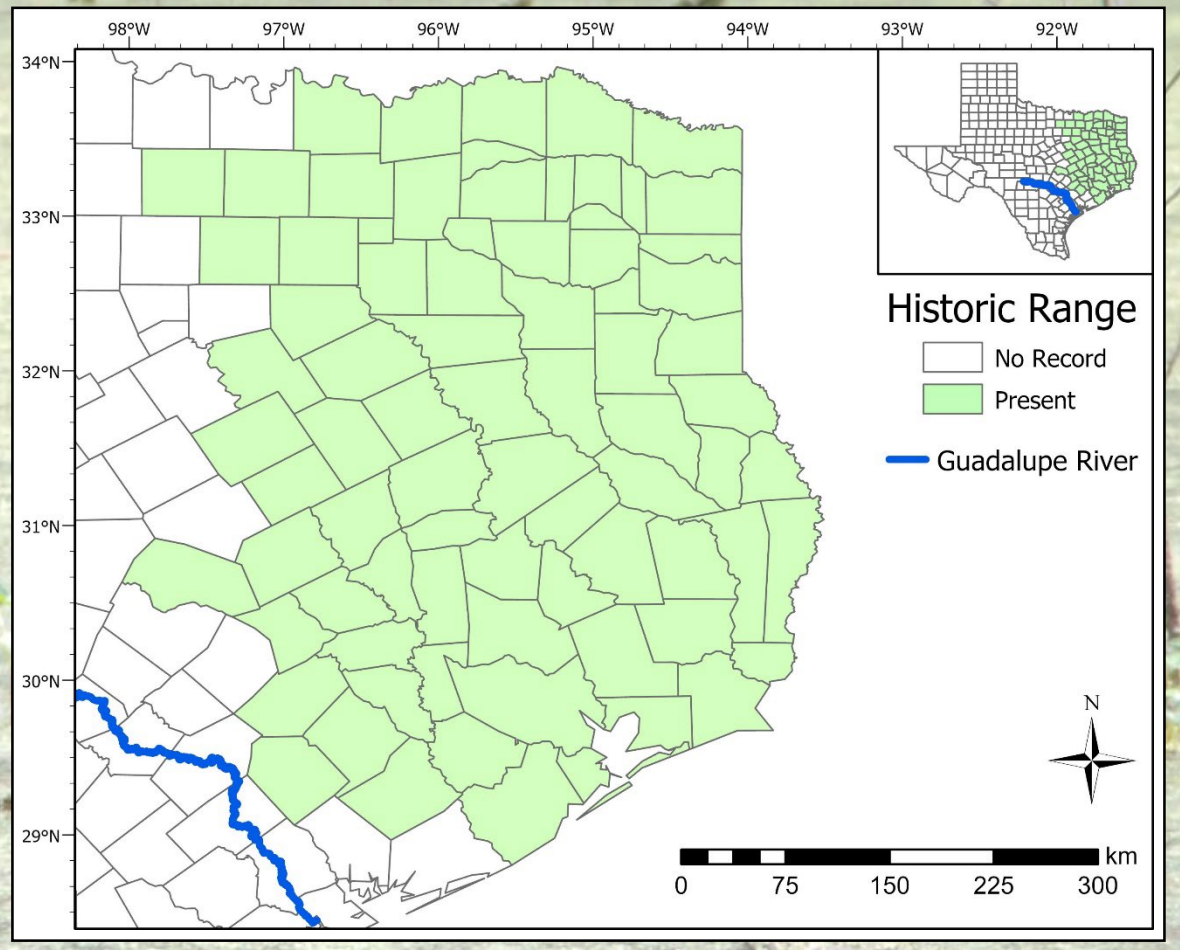
Western Chicken Turtles in Texas

- Historic range extends through east Texas to north of the Guadalupe river basin[9-10]
- Typically found in ephemeral or depressional freshwater wetlands[11-12]
- Shorter life span and smaller population size may increase perception of rarity[13]
- Discrete seasonal activity patterns[14-15]



[9]Dixon 2013
[10]USFWS 2016
[11]Buhlmann et al. 2008
[12]Bowers et al. 2021
[13]Dinkelacker and Hilzinger 2014
[14]McKnight et al. 2015
[15]Bowers et al. 2022

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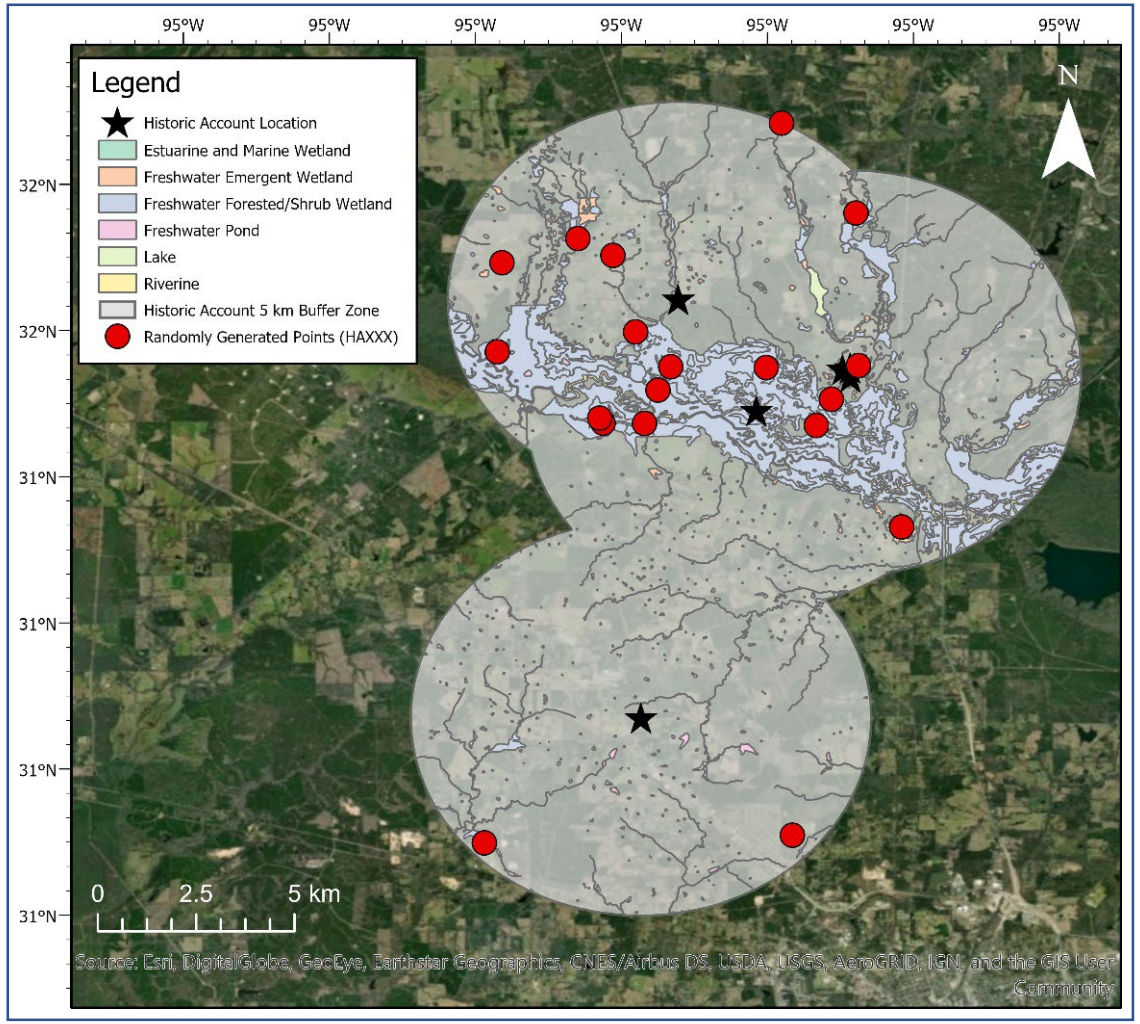
General Study Design

- Randomized locations
  - Historic occurrence data[16-20]
  - Counties in historic range[9-10]
  - Priority wetlands (NWI)[19,21]
- Non-randomized locations
- Seasons[12,15,19] :
  - One event per site per month
  - In-season (late-March to early-July)
  - Out-of-season (August to February)



[16]iNaturalist 2020
[17]VertNet 2020
[18]Adams and Saenz 2011
[19]Ryberg et al. 2016
[20]Franklin et al. 2019
[21]USFWS 2019

https://www.uhcl.edu/environmental-institute/research/current-projects/western-chicken-turtle

https://www.uhcl.edu/environmental-institute/research/publications/

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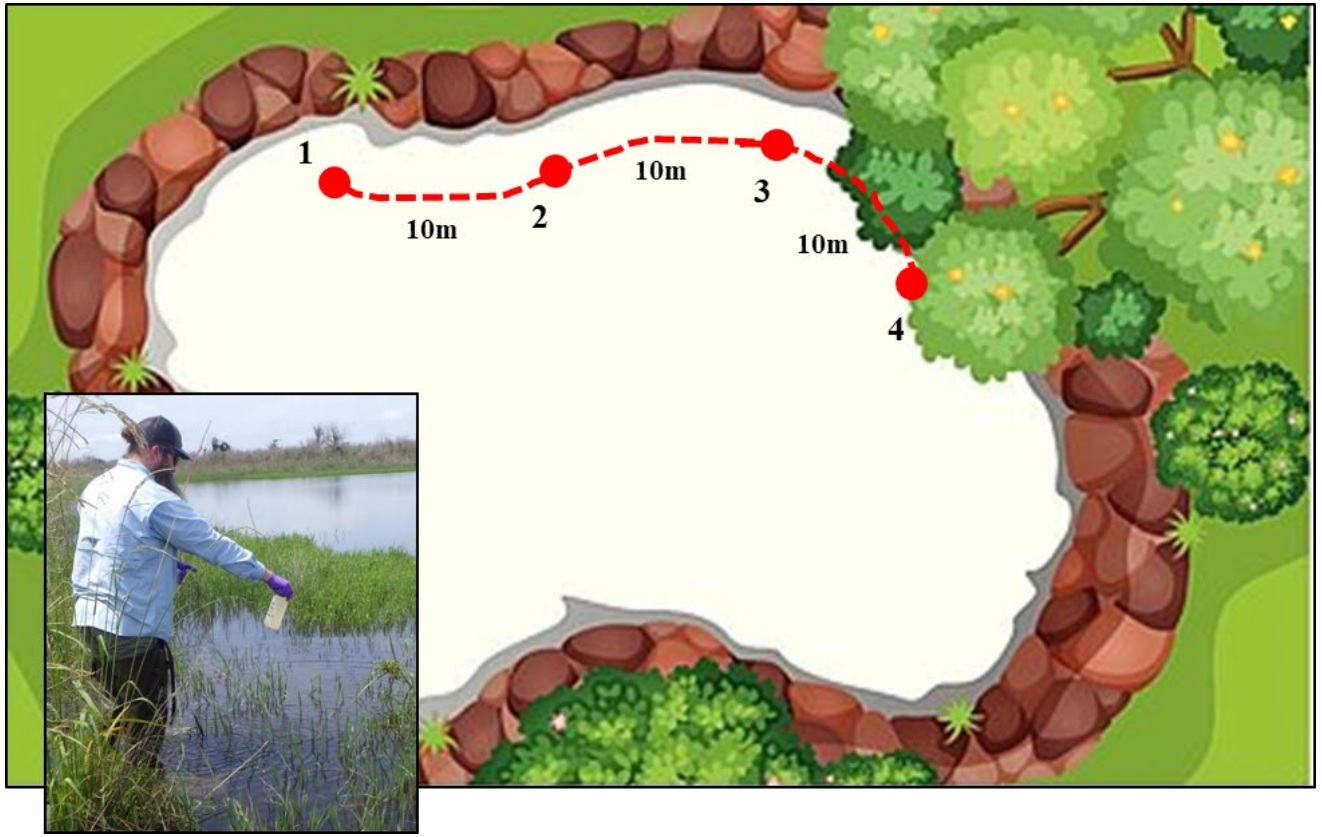
Field Methods

- Water sample collection (4 x 500 mL)
  - Ambient (A) = surface+scum
  - Resuspended sediment (R) = disturbed top 1 cm; collected from plume
- Soil (S) sample collection (3 x 1 tbsp)
- Pre-packaged kits
- Water quality variables
  - Temperature
  - Dissolved Oxygen (mg/L)
  - pH
  - Specific conductivity (µS/cm)



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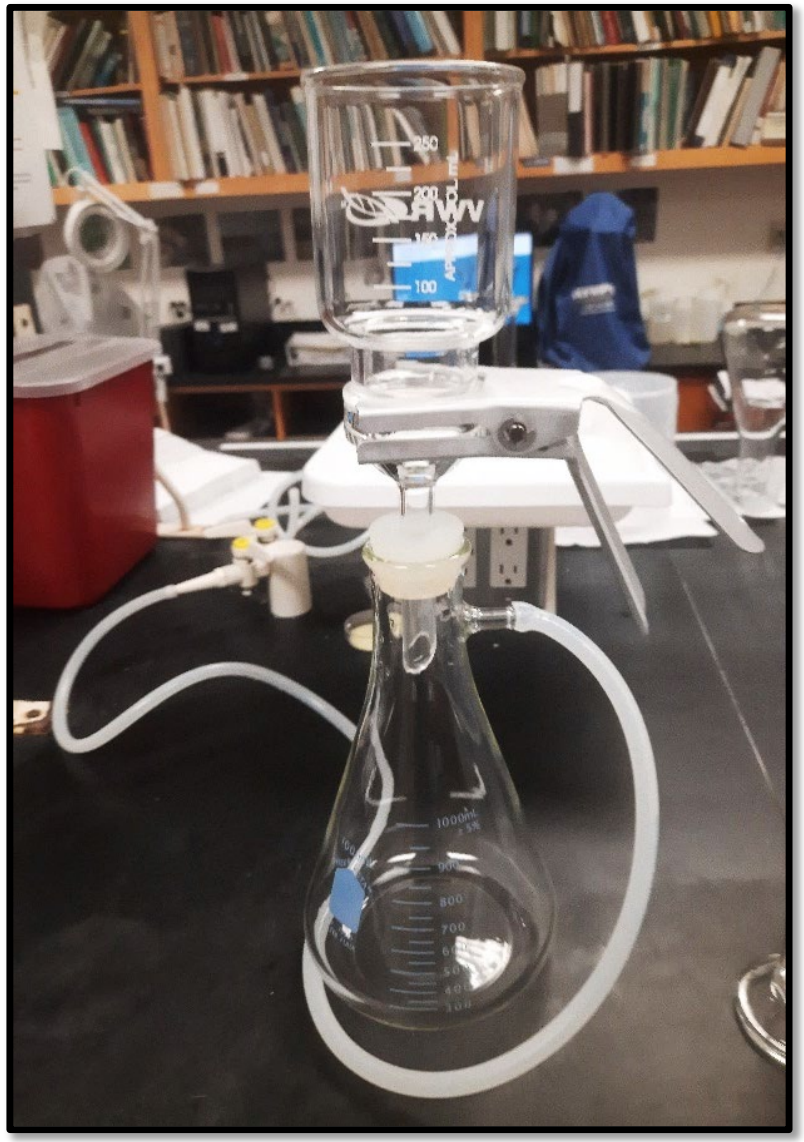
Sample Processing and Lab Methods

- Processed in dedicated lab spaces
- Two filter sizes (cellulose nitrate)
  - A – 0.45 µm and 3.0 µm filters
  - R – 0.45 µm and 3.0 µm filters
  - Soil (no pre-processing)
- Filtered within 72 hours of collection
- Analyzed by Tangled Bank Conservation (qPCR) – 3 replicates
  - Two replicate amplifications = positive
  - One replicate = potential



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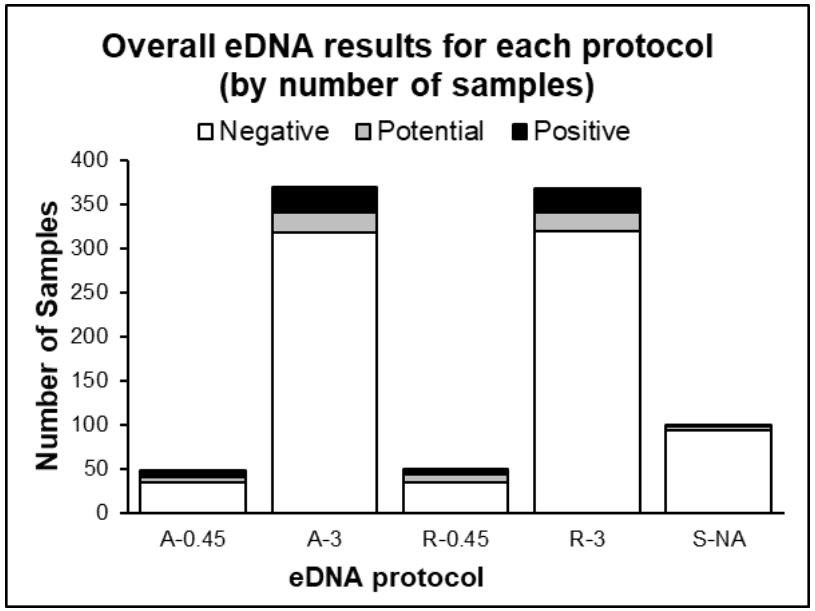
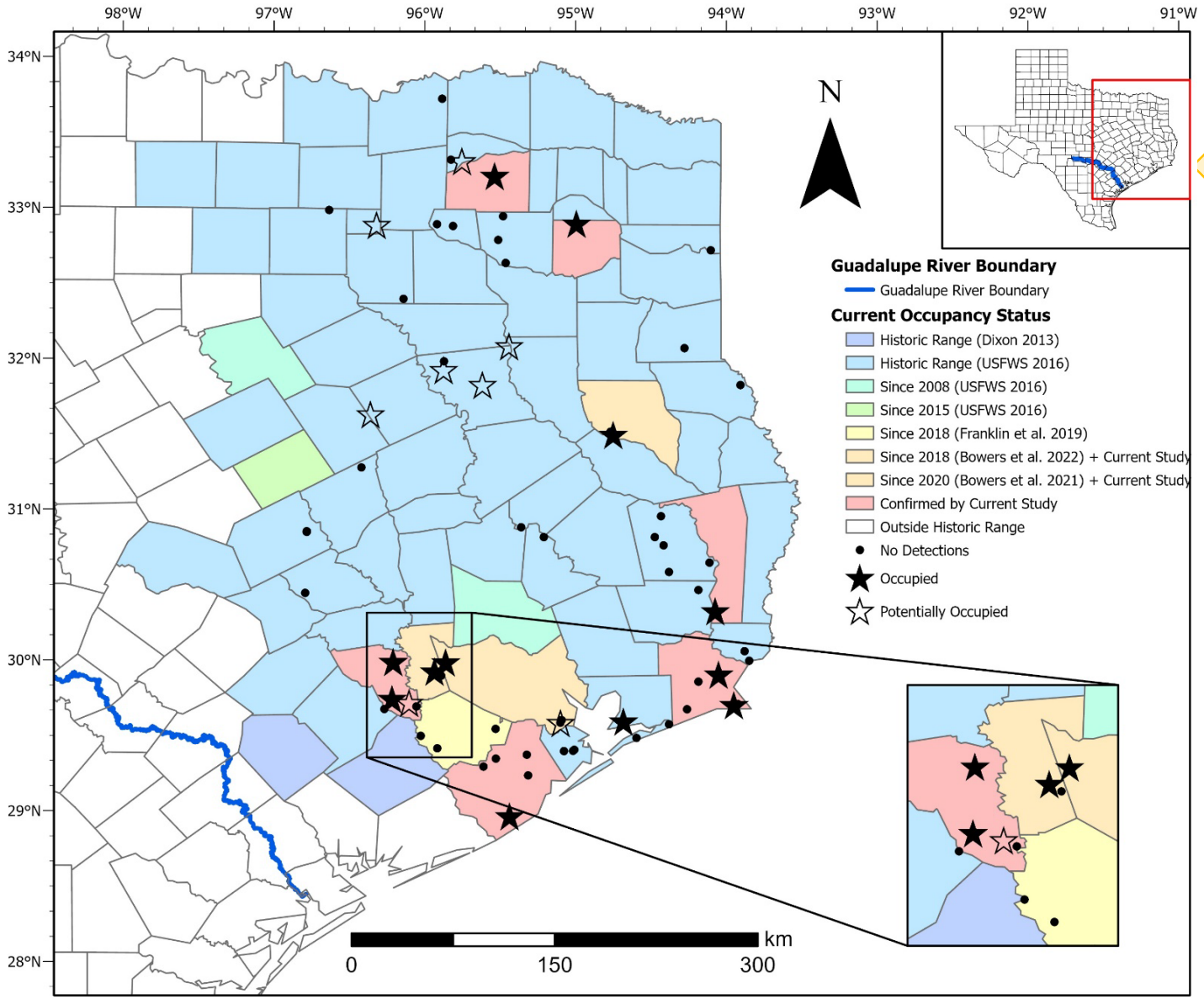
Methods - Data Analyses

- Software
  - Statistical analyses in SigmaPlot v14.5
  - Detectability analyses in R and RStudio (package: *unmarked*)
- Calculations
  - Number of results for each protocol
  - Proportion of results for each protocol
  - Detectability (rho, ρ) for each protocol
  - Overall results from protocol comparison matrix
    - Developed as part of larger study
    - Compares efficiency and efficacy across multiple protocols
    - Considers three broad categories
      - Logistics (9 sub-categories), Statistics (7 sub-categories), Costs (5 sub-categories)

Results – Area Surveyed and Effort

- In-season: 346 events
  66 sites; 33 counties
- Out-of-season: 28 events
  4 sites; 4 counties
- *N* samples = 935
  - A-0.45: *n* = 48; A-3.0: *n* = 369
  - R-0.45: *n* = 50; R-3.0: *n* = 368
  - Soil: *n* = 100





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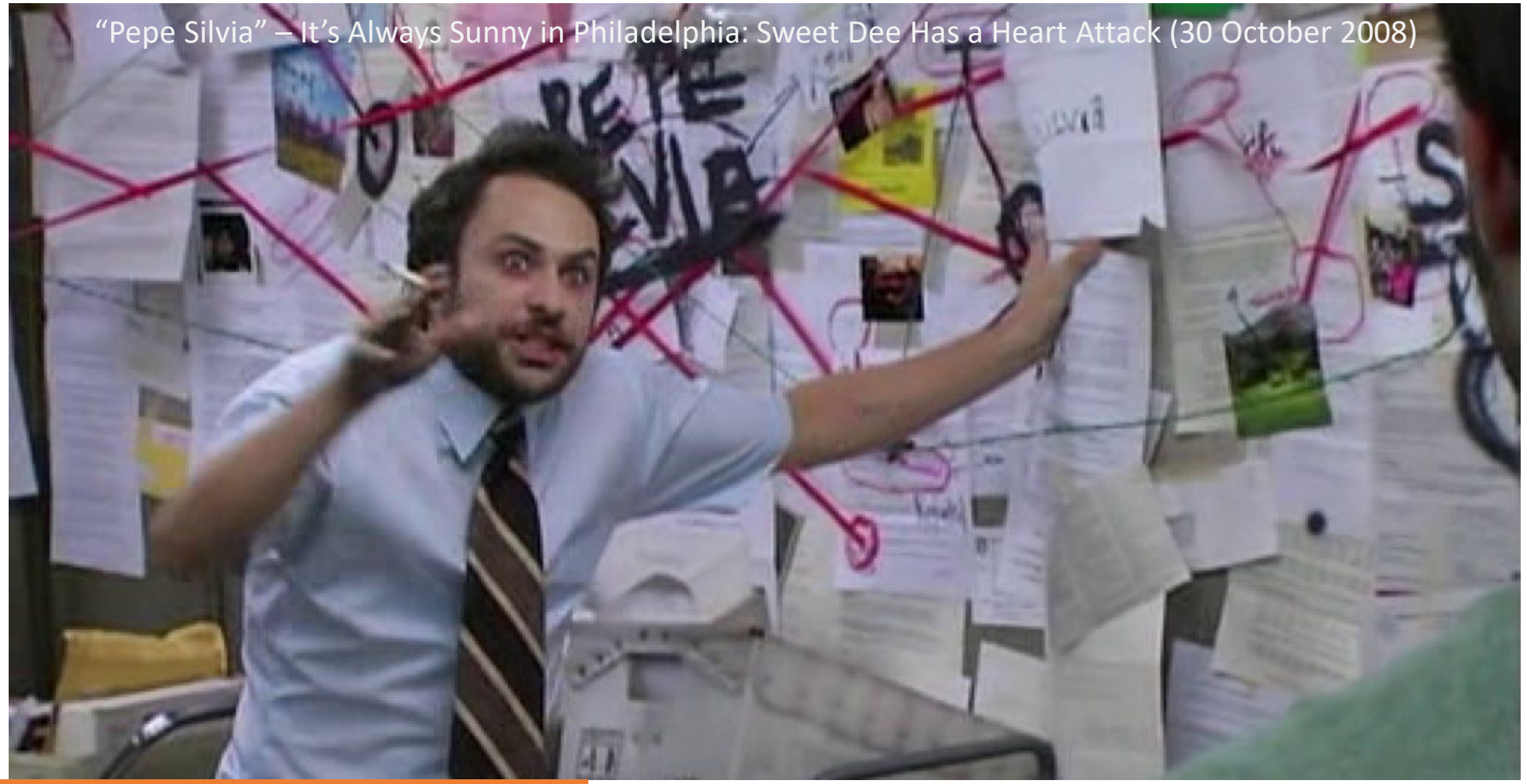
Results



- Number of results for each protocol
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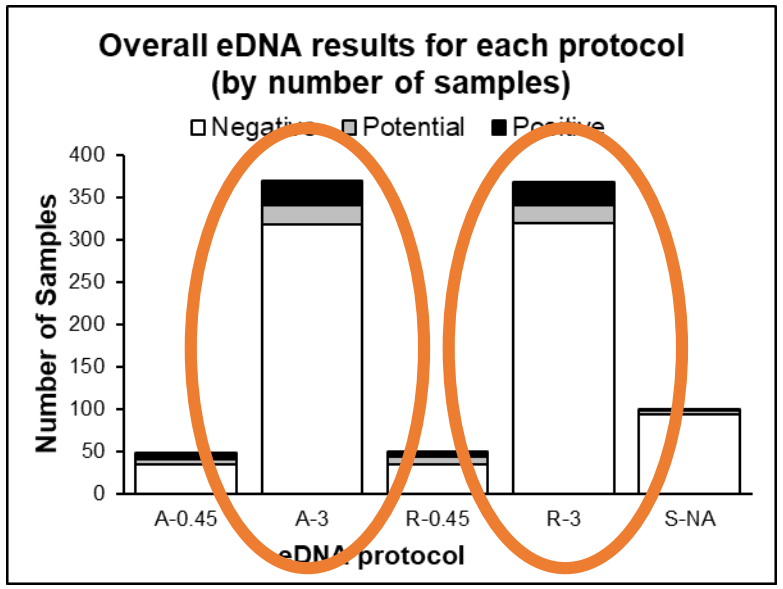
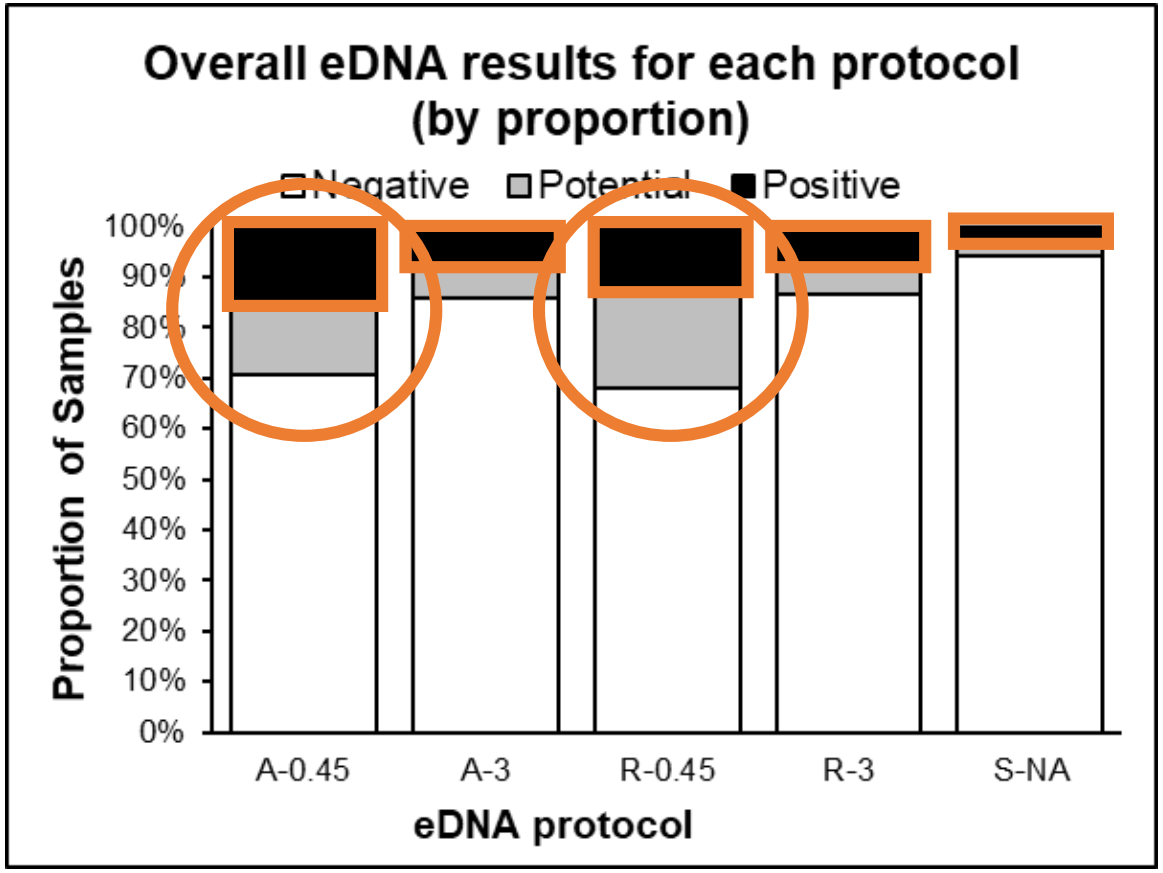
Results – Overall Sample Results (full dataset)

N = 935





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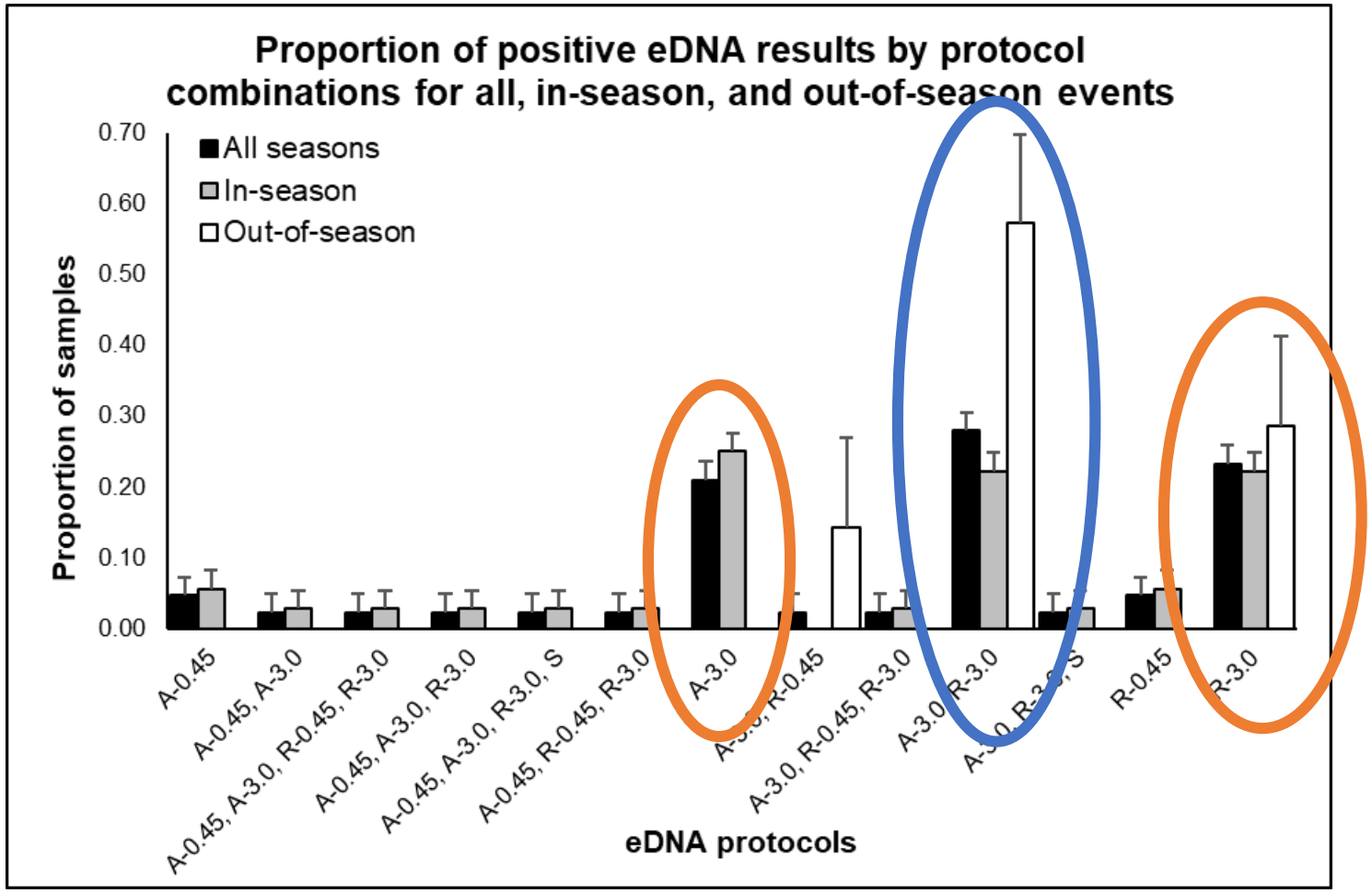
Results – Positive Results Only

All seasons:
n = 43

In-season:
n = 36

Out-of-season:
n = 7



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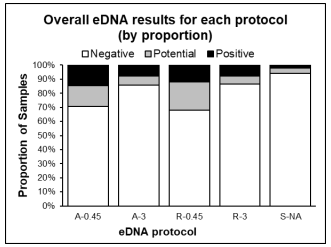

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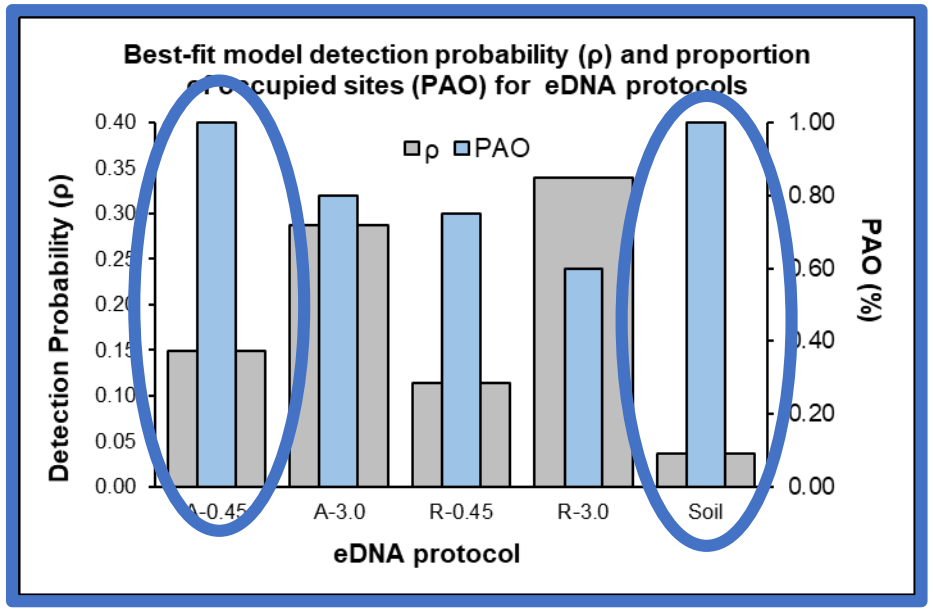
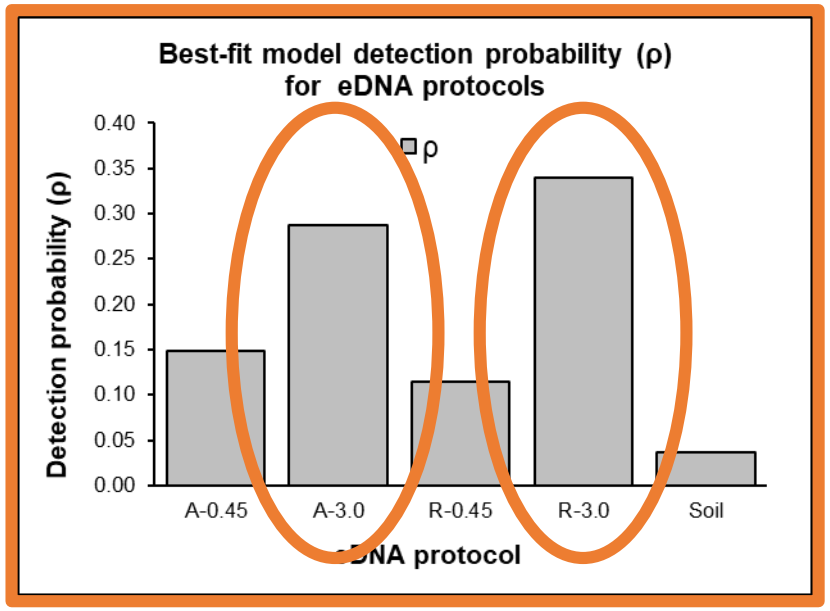
Results – Best-fit Detectability Models (in-season only)

Protocol	*N* Sites	Model Parameters	AIC	ΔAIC	Akaike wt	PAO	95% CI	*t*	*p*	# iterations	ρ
A-0.45	4	Null	43.56	0.00	0.64	1.00	1.00-1.00	47.0	0.548	1000	0.149
A-3.0	10	Ψ (wetland)	105.99	0.55	0.28	0.80	0.80-0.80	80.0	0.213	1000	0.287
R-0.45	4	Ψ (wetland)	32.88	0.00	0.56	0.75	0.75-0.75	35.0	0.263	1000	0.114
R-3.0	10	Ψ (wetland+criteria)	99.34	1.52	0.13	0.60	0.60-0.70	77.1	0.680	1000	0.339
Soil	6	Ψ (habitat)	23.18	2.00	0.23	1.00	1.00-1.00	55.0	0.562	1000	0.036





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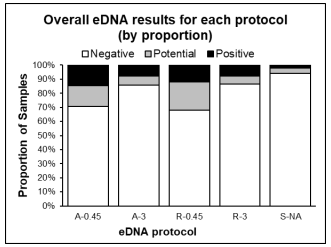

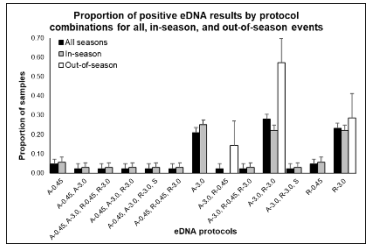

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Results – Protocol Comparison Rubric

Category	Sub-category	Rank Scale	Sub-Category Values				
			A-0.45	A-3.0	R-0.45	R-3.0	Soil
LOGISTICS	Permissions	low = best	0.63	0.63	0.63	0.63	0.63
	Planning	low = best	0.78	0.78	0.78	0.78	0.75
	Difficulty of gear transport	low = best	1.10	1.10	1.10	1.10	0.93
	Difficulty of implementation	low = best	0.53	0.53	0.53	0.53	0.50
	Time and maintenance	low = best	1.47	1.47	1.47	1.47	0.43
	Technical expertise	low = best	1.15	1.15	1.15	1.15	0.59
	Performance variability	low = best	0.58	0.58	0.58	0.58	0.61
	Potential for failure	low = best	0.75	0.75	0.75	0.75	0.69
	Resolution	low = best	0.81	0.81	0.81	0.81	0.86
STATISTICS	Number of personnel (N_{pers})	low = best	8	8	8	8	7
	Number of sites (N_{sites})	high = best	4	4	4	4	6
	Detection probability (ρ)	high = best	0.1490	0.2870	0.1140	0.3390	0.0360
	"Catch" per unit effort (CPUE)	high = best	0.5833	0.9739	0.4800	0.9825	0.1412
	Detection proportion (Det%)	high = best	15%	24%	12%	25%	2%
	Geographic coverage (G_{cov})	high = best	0.00017%	0.00017%	0.00017%	0.00017%	0.00001%
COSTS	Stages of analysis (N_{stages})	low = best	10	10	10	10	10
	Start-up costs (C_{start})	low = best	$2,500	$2,500	$2,500	$2,500	$1,550
	Cost per event (C_{event})	low = best	$896	$888	$1,126	$1,115	$529
	Time (pre-field) (T_{pre})	low = best	0.25	0.25	0.25	0.25	0.25
	Time (field) (T_f)	low = best	0.25	0.25	0.25	0.25	0.17
	Time (post-field) (T_{post})	low = best	0.38	0.37	0.62	0.61	0.16

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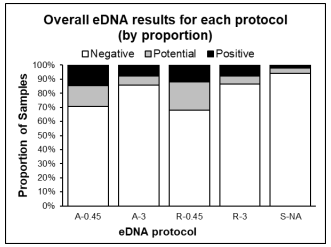

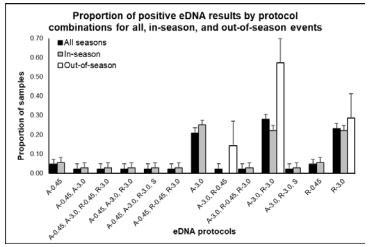

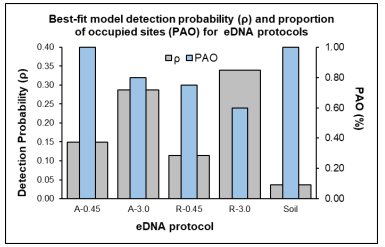

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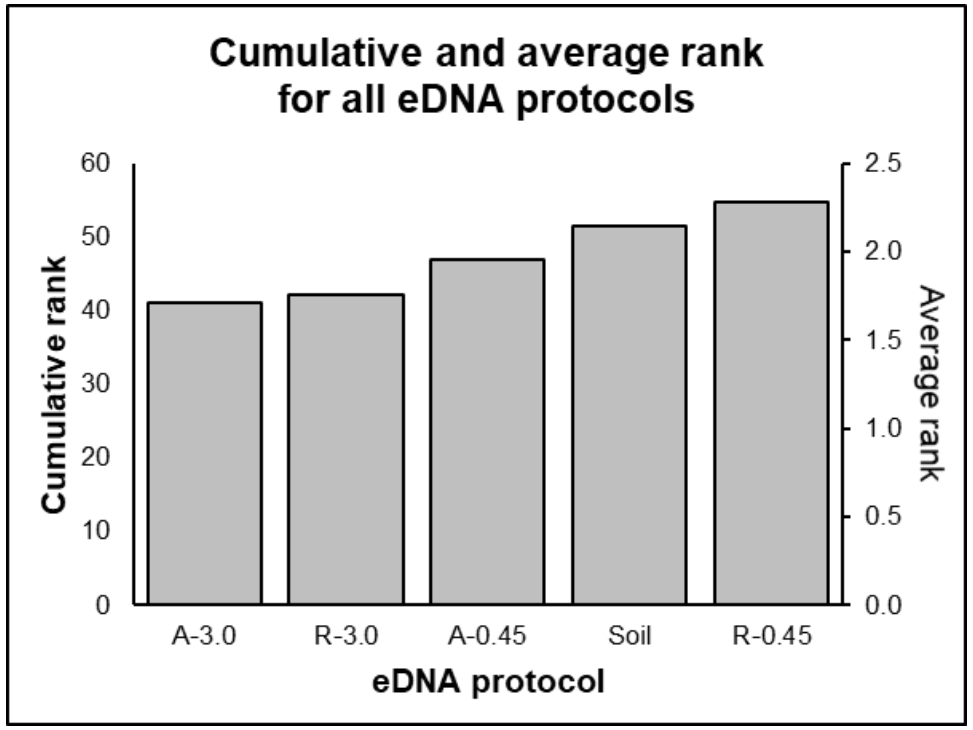
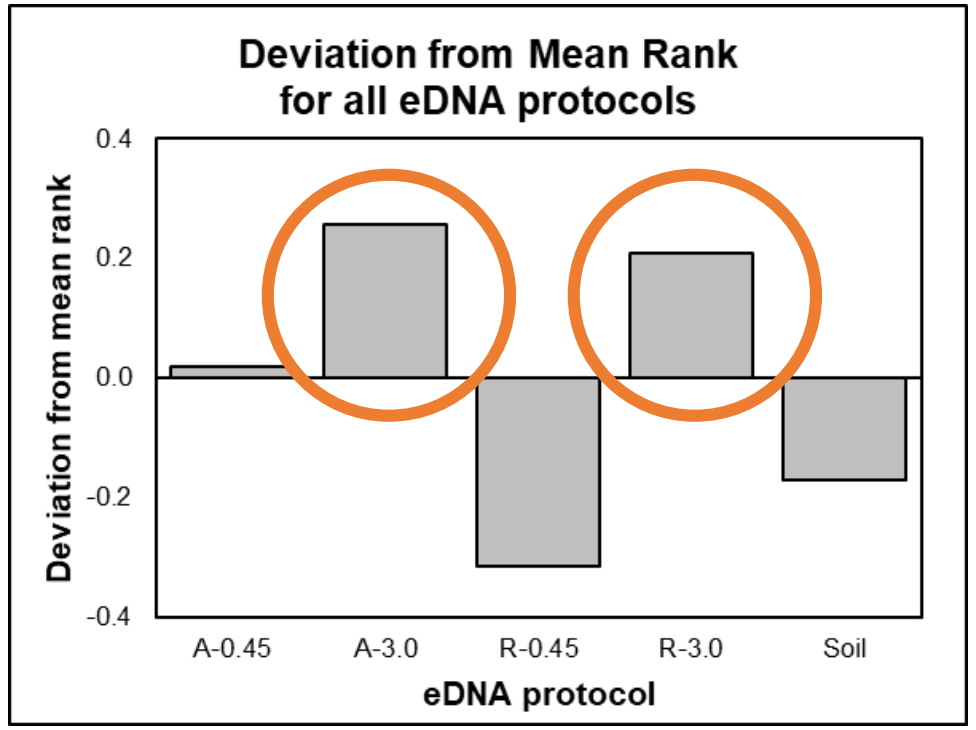
Results – Protocol Comparison Rubric Ranks





Kruskal-Wallis One-way ANOVA on Ranks

H = 4.585, df = 4, p = 0.333

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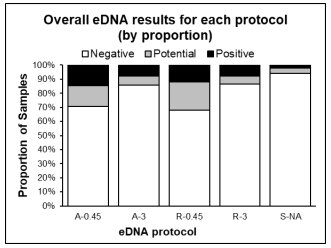

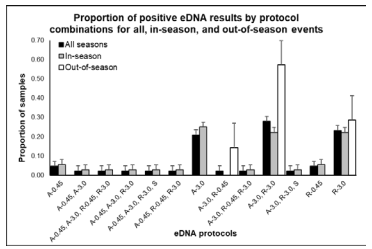

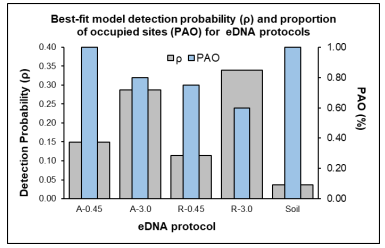

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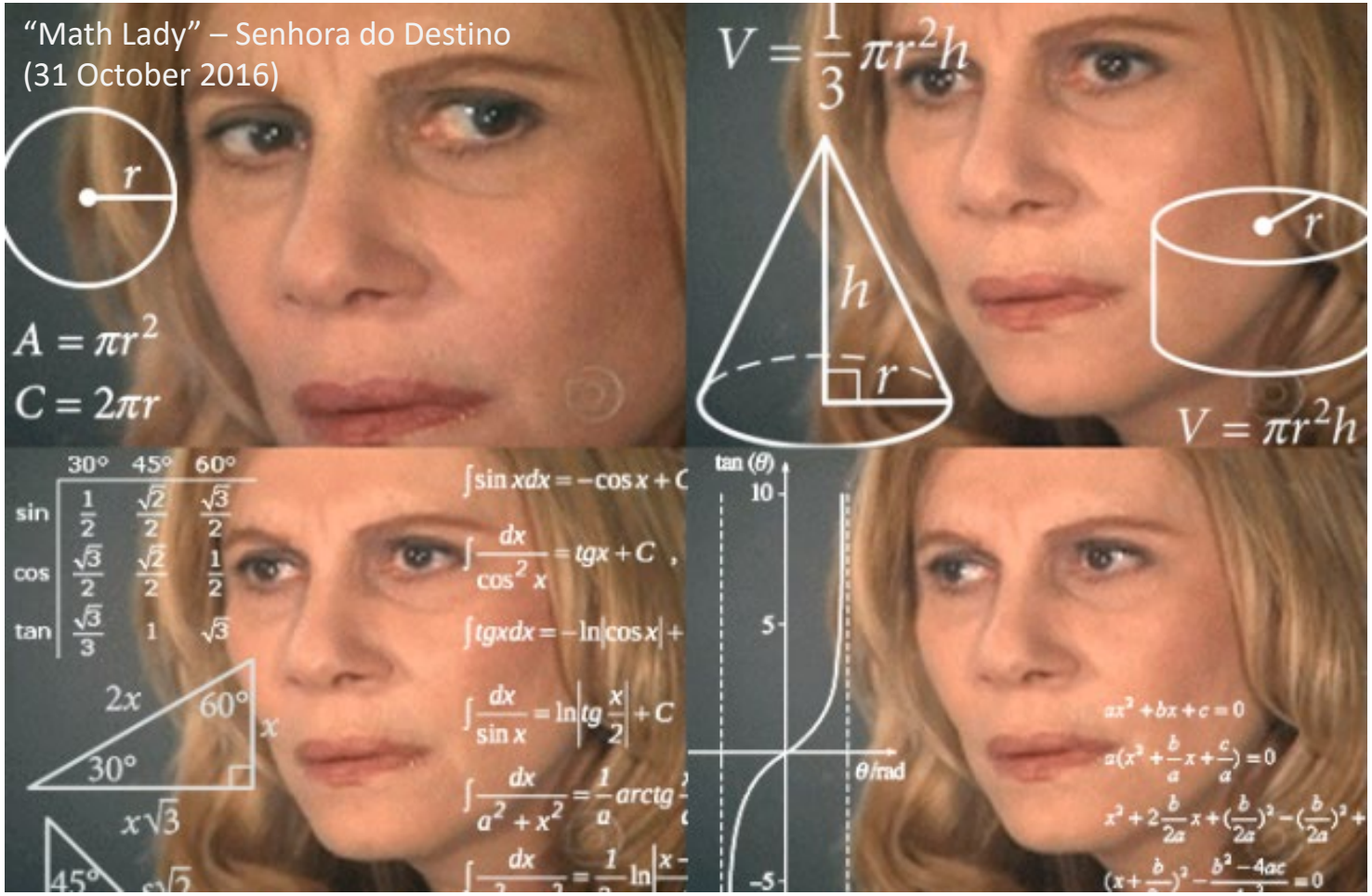
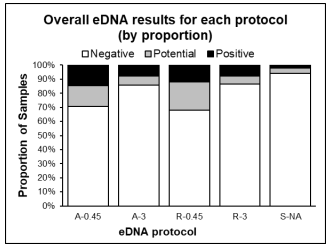
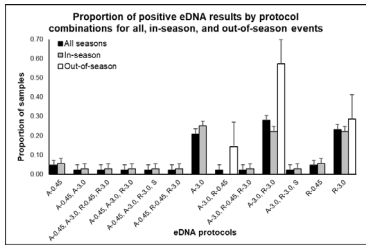
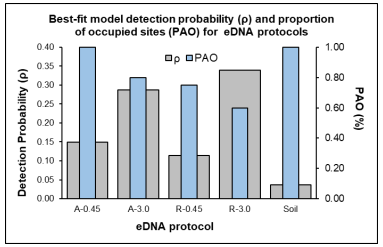
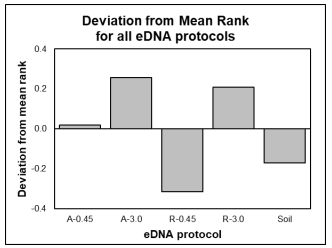
Discussion











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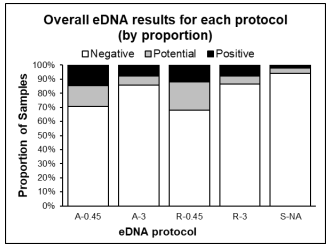
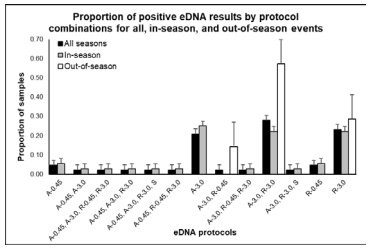
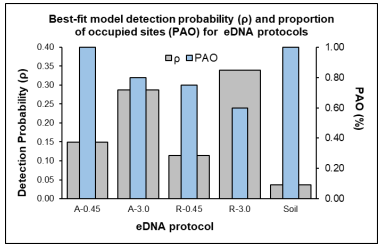
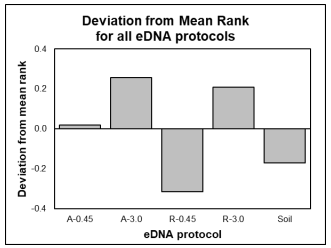
Discussion

- Best recommendation: combination of ambient and resuspended samples filtered with 3.0 µm filter (A-3.0 and R-3.0) – *not* soil samples
- Questions about consistency in data
  - 28 events with positive eDNA detections and no confirmation from another protocol
  - Nine events with positive eDNA detections and confirmation from another protocol
  - Three events with detections using other protocols but no positive eDNA results
  - Two instances of soil samples collected at the location of a WCT – no eDNA detection
- Factors impacting eDNA residency or degradation rates[22-26]:
  - Holding time - persistence decreases after 96 hours since deposition
  - Exposure to UV radiation, especially during drought (year 1)
  - Dilution, especially from heavy rain or flooding (year 2)
  - Increased presence of inhibiting compounds
  - Seasonal activity of target species









[22]Piaggio et al. 2014
[23]Barnes and Turner 2016
[24]de Souza et al. 2016
[25]Seymour et al. 2018
[26]Stewart 2019

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Future Plans and Recommendations

- Continued evaluation of water quality impacts to eDNA detectability and detection rates
- Final report for larger Western Chicken Turtle (WCT) project will be published in March
- Recommendations for next steps:
  - Evaluation of eDNA detection at locations specifically known to be occupied by WCT, especially in off-season
  - Increased number of composite samples
  - Evaluation of eDNA detection using larger pore size filters or different filter types (e.g., not cellulose nitrate)
  - Evaluation of eDNA persistence and time frame(s) needed to maximize DNA amplification (all steps of the process)

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Questions: gordon@uhcl.edu

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