

Environmental DNA metabarcoding survey of non-fish vertebrates at the Black Island CA

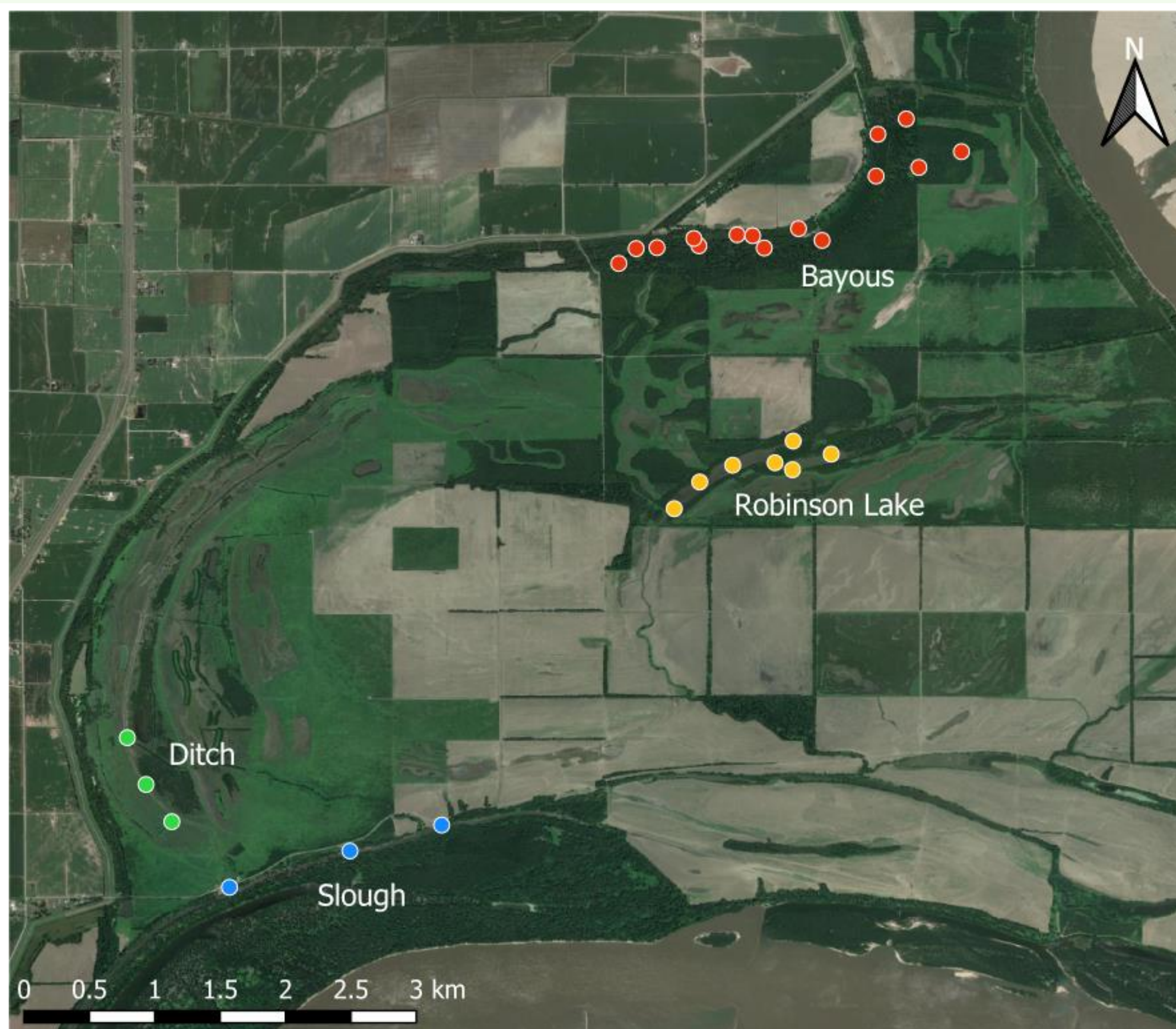
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Introduction

Environmental DNA (eDNA) metabarcoding has been widely used in aquatic systems to sample and characterize fish communities (Yao et al. 2022). Mitochondrial DNA genes are most often targeted for eDNA sampling because they have favorable properties that include high copy number in cells and gene sequences with highly variable regions flanked by conserved regions. Commonly used polymerase chain reaction assays that target fish (e.g. Miya et al. 2015) also effectively sample other vertebrate taxonomic groups. In this study we utilized sequence data collected to assess fish communities to also explore the detection of other vertebrate taxonomic groups.

Study Sites

- In Spring and Fall 2022, Ludwig et al. (2023) sampled aquatic environments at Black Island Conservation Area, a wetland complex along the Mississippi River in Missouri's Bootheel.
- A total of 28 sites among these habitats were selected for fish community sampling and 3 water samples were collected at each site.



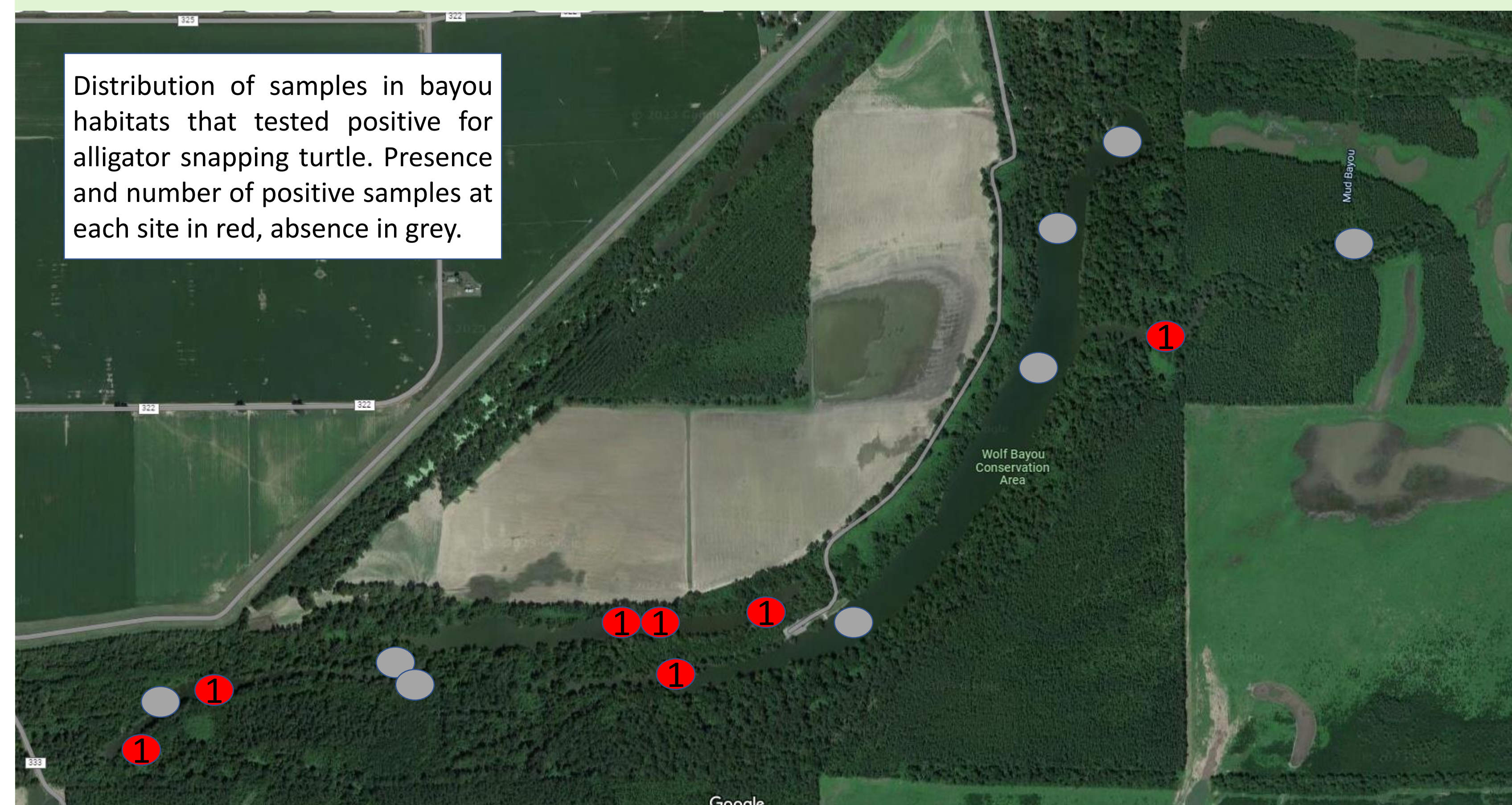
Methods

- Water sample collection, DNA extraction, PCR amplification, and eDNA sequencing were performed for fish community sampling.
- Fish species were identified from Illumina DNA sequences using the Barque bioinformatics pipeline, and a DNA sequence database containing all fish species known to occur in Black Island Conservation Area habitats.
- We explored DNA sequences that were not identified as fish. Sequences were BLAST searched in GenBank to identify additional species.
- All identified additional species were added to the fish database and Barque was re-run.
- We summarized the distributions of all non-fish vertebrate species detected.

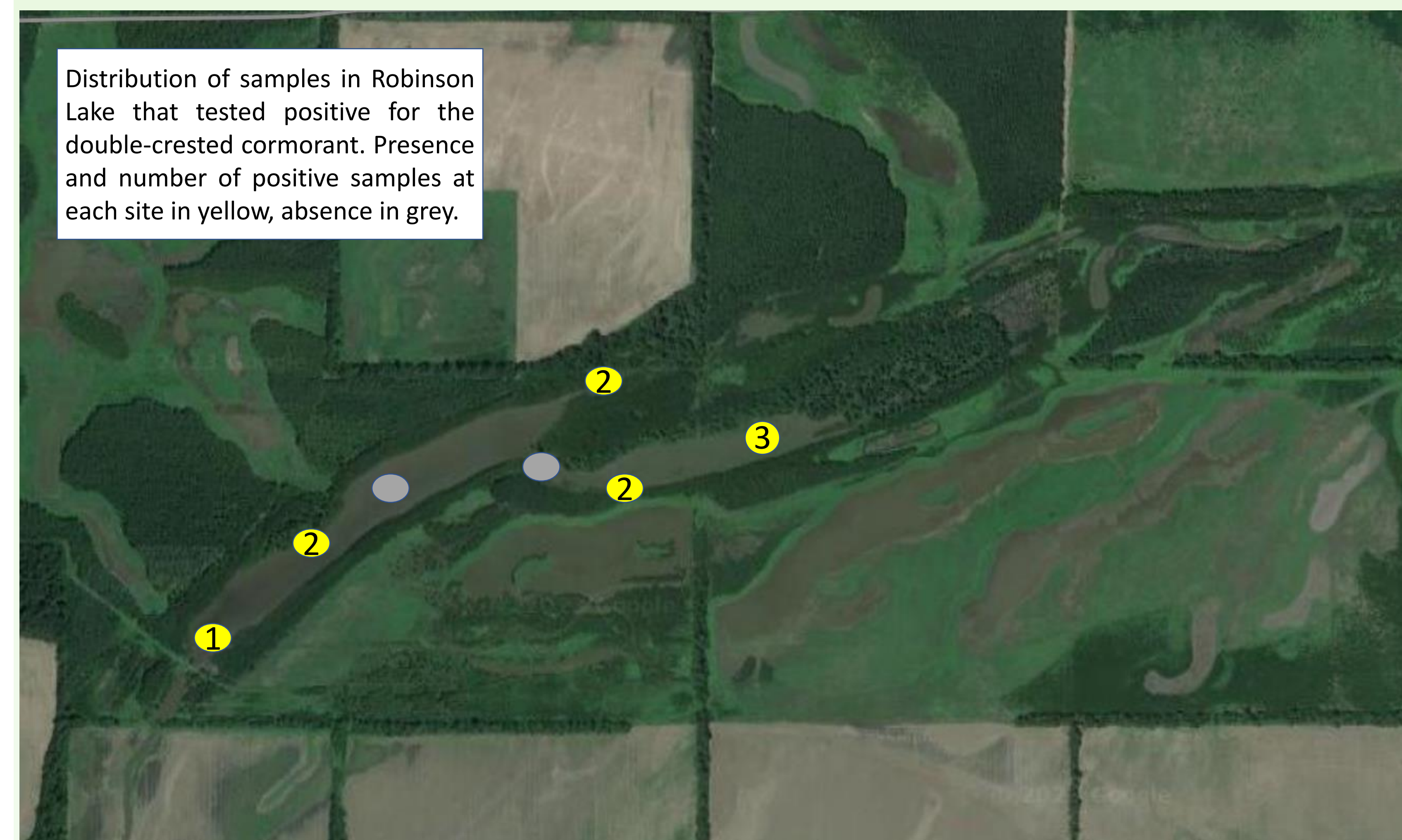
Chord16s Primers

Species	Chord16sF	Chord16sR
Oligonucleotide primer sequences	5' - AGACGAGAAGACCCTRTGGAGCT - 3'	5' - CCTNGGTCGCCCCAAC - 3'
Fowler's toad (<i>Anaxyrus fowleri</i>)	AGACGAGAAGACCCTATGGAGCT	CCGCGGTCACCCCAAC
Green frog (<i>Lithobates clamitans</i>)	AGACGAGAAGACCCTATGGAGCT	CCGTGGTCCCCCAAC
Southern leopard frog (<i>Lithobates sphenoccephalus</i>)	AGACGAGAAGACCCTATGGAGCT	CCGAGGTCACCCCAAC
Grey heron (<i>Adea cinerea</i>)	AGACGAGAAGACCCTATGGAGCT	CCAAGGTCGCCCCAAC
Wild turkey (<i>Meleagris gallopavo</i>)	AGACGAGAAGACCCTGTGGAAGCT	CCAAGGTCGCCCCAAC
American beaver (<i>Castor canadensis</i>)	AGACGAGAAGACCCTATGGAGCT	CCGAGGTCACCCCAAC
Deer mouse (<i>Peromyscus leucopus</i>)	AGACGAGAAGACCCTGTGGAAGCT	CCGAGGTCACCCCAAC
Alligator snapping turtle (<i>Macrochelys temminckii</i>)	AGACGAGAAGACCCTATGGAGCT	CCGAGGTCGCCCCAAC
Common water snake (<i>Nerodia sipedon</i>)	AGACCAGAAGACCCTGTGGAAGCT	CCAAGGTCGCCCCAAC

From the 16s ribosomal RNA, the forward and reverse primer sequences for the Chord16s primers (Deagle et al. 2009) are compared for species present at Black Island Conservation Area. Mismatch sites are highlighted in bold. The primer sequences are a close match to 16s sequences in all vertebrates that could be found in the Black Island Conservation Area habitats.



Distribution of samples in bayou habitats that tested positive for alligator snapping turtle. Presence and number of positive samples at each site in red, absence in grey.



Distribution of samples in Robinson Lake that tested positive for the double-crested cormorant. Presence and number of positive samples at each site in yellow, absence in grey.

Species detected

Species	Bayou (87)	Slough (15)	Lake (27)	Ditch (8)	Total (137)
Blanchard's cricket frog (<i>Acris blanchardi</i>)	1	0	0	0	1
American toad (<i>Anaxyrus americanus</i>)	5	3	0	2	10
Fowler's toad (<i>Anaxyrus fowleri</i>)	1	2	0	3	6
American green tree frog (<i>Dryophytes cinereus</i>)	1	0	1	0	2
Green frog (<i>Lithobates clamitans</i>)	2	0	0	0	2
American bullfrog (<i>Lithobates catesbeianus</i>)	0	0	1	0	1
Southern leopard frog (<i>Lithobates sphenoccephalus</i>)	5	4	0	0	9
Grey heron (<i>Adea cinerea</i>)	12	0	3	0	15
Double-crested cormorant (<i>Phalacrocorax auritus</i>)	0	0	10	0	10
Common grackle (<i>Quiscalus quiscula</i>)	7	0	1	0	8
Cattle (<i>Bos taurus</i>)	18	8	13	4	43
American beaver (<i>Castor canadensis</i>)	44	8	13	2	67
Cat (<i>Felis catus</i>)	9	3	8	1	21
Eastern wood mouse (<i>Neotoma floridana</i>)	4	2	4	1	11
White-tailed deer (<i>Odocoileus virginianus</i>)	13	1	3	0	17
Deer mouse (<i>Peromyscus leucopus</i>)	12	2	2	1	17
Raccoon (<i>Procyon lotor</i>)	16	3	4	1	24
Hog (<i>Sus scrofa</i>)	2	0	4	3	9
Spiny softshell turtle (<i>Apalone spinifera</i>)	8	4	7	2	21
Alligator snapping turtle (<i>Macrochelys temminckii</i>)	7	0	0	0	7
Pond slider (<i>Trachemys scripta</i>)	38	9	18	4	69
Ouachita map turtle (<i>Graptemys Ouachitensis</i>)	29	6	3	3	41
River cooter (<i>Pseudemys Concinna</i>)	54	3	1	1	59

Summary of detections of non-fish species. For each habitat type, the number of positive water samples is indicated for each species. Total number of samples in each habitat type is indicated in parentheses.

Discussion

- In total, we detected seven amphibians, three birds, eight mammals, and five reptile species. All native species are known to be present in Black Island Conservation Area. The most common species detected were several species of turtles and beaver.
- Many of the species are common, but some are of conservation interest. The alligator snapping turtle was detected in multiple samples throughout the bayou complex but not in any of the other three habitats. Positive samples occurred in shallow sites and creek inlets in both May and October.
- The double-crested cormorant was abundant in Robinson Lake but absent from all other habitats. All of the samples were from May, when Robinson Lake may have hosted a rookery for the species.
- Domestic/feral species detected included cattle, hogs and cats. The source of these detections was not clear.
- Notably, there were no detections of water snakes despite observation of an abundance of snakes on both collecting dates. Snakes may not shed as much DNA into the environment as some of the other species rendering them more difficult to detect.
- Overall, this study shows that eDNA surveys designed to sample fish communities can also be used to gain information about other vertebrate species distributions.

Acknowledgements

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References

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- Miya et al. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA fishes: detection of more than 230 subtropical marine species. *Royal Society Open Science*, 2:150088
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