Environmental DNA metabarcoding detects mammal use of stock tanks and natural springs on the Prescott National Forest

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Introduction

Recent research has shown the viability of environmental DNA (eDNA) analysis as a tool for identifying species presence in aquatic ecosystems (Valentini et al. 2016). Because of the scarcity of water in the desert Southwest, natural springs and stock tanks provide an important water resource for wildlife.

In this study, we examined whether 16S rRNA mtDNA vertebrate metabarcoding protocol could be used to detect mammal use of springs and stock tanks in the Prescott National Forest.

Methods

During summer 2019, we collected water samples at 5 natural springs and 3 stock tanks. Triplicate 250 mL samples were collected and filtered (0.45 μm CN membrane) at sites and were transported on ice to the Forensic Biology lab at ERAU.

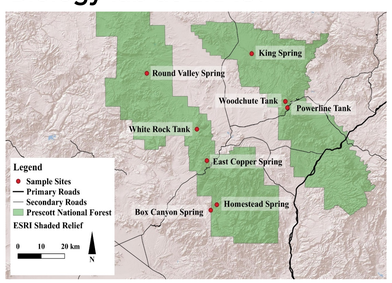

Fig. 1. Map of sample sites at natural springs and stock tanks on the Prescott National Forest.

In the lab, eDNA was extracted with a Qiagen DNeasy Blood & Tissue kit and PCR inhibitors were removed. Vences et al. (2016) primers were used to amplify a portion of the *16S rRNA* mtDNA gene. PCR products were cleaned using a MagBind TotalPure NGS kit and verified via gel electrophoresis. A second PCR was performed using the Illumina Nextera XT Index kit, and sequencing was completed using an Illumina FGx Forensic Genomics System. Sequence results were quality filtered and clustered into operational taxonomic units (OTUs) at 97% similarity using USEARCH. OTUs were then identified using the GenBank nucleotide database BLAST tool.

Results

Of the 1.9 million sequences analyzed, 1.85 million belonged to invertebrate species. Most of these sequences belonged to waterfleas (e.g., *Daphnia*) which suggests that the Vences et al. (2016) primers are not vertebrate-specific. We had successful amplification of vertebrate eDNA at 5/8 sites that included detections of 1 amphibian, the American Bullfrog, 1 bird, the Mallard, and 5 mammals. DNA from the Rhesus Macaque served as our positive control and was detected only in this sample.

Fig. 2. (A) A sample being collected at Box Canyon Spring, (B) the sample filtration set-up in the field, (C) a sample filter, (D) filter in a sterile tube for transport to the lab, and (E) Completing eDNA extraction in the lab.

Table 1. Vertebrate species detected in samples from stock tanks and natural springs. The values represent the total number of eDNA sequences detected at sample sites. For * see Limitations.

Common name	Scientific name	Field Negative 1	Field Negative 2	Field Negative 3	Positive Control	East Copper Spring	White Rock Tank	King Spring 1	King Spring 2	Round Valley 1	Round Valley 2	Woodchute Tanks	Powerline Meadows	Box Canyon 1	Box Canyon 2	Homestead Spring
American Bullfrog	*Lithobates catesbeiana*	0	0	0	0	0	0	3	13	136	9	9	0	0	0	0
Mallard	*Anas platyrhynchos*	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
Cattle	*Bos taurus*	0	0	0	0	4824	0	20	0	0	297	720	0	0	0	0
Elk	*Cervus elaphus*	0	0	0	0	0	0	0	0	0	0	586	0	0	0	0
Mule Deer*	*Odocoileus hemionus*	0	0	0	0	188	0	0	0	0	0	0	0	0	0	0
Arizona Gray Squirrel*	*Sciurus arizonensis*	0	0	0	0	0	18843	0	0	0	0	0	0	0	0	0
American Black Bear	*Ursus americanus*	0	0	0	0	0	519	0	0	0	0	0	0	0	0	0
Rhesus macaque	*Macaca mulatta*	0	0	0	895	0	0	0	0	0	0	0	0	0	0	0

Limitations

- Non-target amplification of invertebrate eDNA means fewer vertebrate eDNA detections.
- The Mule Deer OTU was 100% match to the Mule Deer and the White-tailed Deer *Odocoileus virginianus*, suggesting shared haplotypes and the inability to distinguish between these species with these primers.
- A reference 16S rRNA sequence for the Arizona Gray Squirrel is absent from GenBank, so this is a hypothesis based on the OTU being a 96% match to other members of the Genus *Scuirus* and the tank being in a riparian area.

Conclusions

Amplification of invertebrate eDNA indicates that these primers may be better suited to targeting animals, rather than specifically vertebrate species. Our plan is to re-analyze eDNA from these samples using a mammal-specific primer set developed by Ushio et al. (2017) to see if we recover more species.

Although this was a pilot study, our results compliment a growing body of literature that suggests that eDNA metabarcoding may be a viable tool for monitoring mammal use of aquatic ecosystems (Klymus et al. 2017, Ushio et al. 2017, Ruppert et al. 2019).

Literature cited

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Acknowledgments

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