



B84 Using Environmental DNA (eDNA) and Vertebrate Metabarcoding to Characterize Biological Communities: A New Method for Conservation Genetics and Wildlife Forensics

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Learning Overview: After attending this presentation, attendees will better understand the impact of vertebrate metabarcoding on wildlife forensics and wildlife management plans for threatened and endangered species, as well as those of conservation concern.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a novel technique for genetic species identification of unknown vertebrate biological samples for both conservation and law enforcement efforts in combating wildlife crimes.

Illegally poached wildlife products are estimated to bring \$20 billion in annual revenues from black market trading and has been established as a source of funding for terrorism. Poaching causes a dramatic drop in biodiversity, which leads to a further decline of the ecosystem, especially if keystone species, such as elephants, are targeted. Historically, surveys for fish and wildlife have required a significant number of person-hours in the field, using methods that can be stressful to the species being observed. However, the analysis of eDNA, left behind by organisms in water, soil, and air, is revolutionizing biologists' ability to rapidly assess biodiversity. Vertebrate metabarcoding represents an advance in high-throughput DNA sequencing that offers the wildlife community a dramatic improvement in the process of species identification, as it requires only one primer set for all samples, and not species-specific primers.

In this study, the application of the 16S rRNA mitochondrial gene for vertebrate eDNA metabarcoding was explored in Arizona's Verde River, home to more than 50 species of fish and wildlife that are endangered, threatened, or of conservation concern. Replicate water samples of 250mL volumes were collected consecutively from two sites along the river, both prior to and during the annual monsoon season to ensure a robust method in the face of increased organic matter during the monsoon season. Samples were vacuum filtered to collect eDNA onto cellulose nitrate filters. DNA was extracted, amplified by polymerase chain reaction, and libraries were prepared for sequencing on an Illumina® MiSeq® FGx Forensic Genomics System. Sequences were compared to the National Center for Biotechnology Information (NCBI) nucleotide database using the Basic Local Alignment Search Tool (BLAST).

Members of every vertebrate taxon were identified from sequences, including fish, reptiles, amphibians, birds, and mammals. All taxa that were detected corresponded to those known to occur in the Verde River riparian corridor, but there were also new discoveries at the species level.

This technique represents a novel method to characterize vertebrate communities from water samples that is more efficient than quantitative Polymerase Chain Reaction (qPCR), as it does not require species-specific primers, and less stressful to species than traditional survey methods, as no capturing of wildlife is required. Greater than 25 species from diverse taxa were identified with a single water sample collected in a few minutes. The use of eDNA can supplement or potentially someday replace more invasive techniques for surveying wildlife. This method can be used in conservation efforts by wildlife managers to track migration patterns, invasive species, and those of conservation concern, as a few examples. Examples of how this technique could also be applied to wildlife forensics will be discussed. This technique provides the wildlife community with a powerful analytical tool that has wide applicability across conservation and forensic disciplines and will increase the efficiency of investigations and surveys, while decreasing overall cost.

eDNA, Vertebrate Metabarcoding, Wildlife Conservation and Forensics