

////Title: Improving Wildlife Surveys through Environmental DNA

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To understand how human activities impact wildlife, biologists need to know which species are present within a given area. Traditional methods of surveying species are often labour intensive, time consuming, and expensive. However, recent advancements in genetic analysis may provide powerful new survey tools to complement existing methods. Through her research, Dr Katy Klymus (Kay-tee Kligh-miss) at the US Geological Survey tests the efficacy of one such tool, environmental DNA metabarcoding, for identifying wildlife affected by contaminated water sources.

////Main text:

The scientific field of ecotoxicology examines how environmental contaminants, such as the run-off from uranium mining activities, impact wildlife species, natural communities and entire ecosystems. Contaminants often move through the food chain, affecting multiple species within an ecosystem. Thus, surveys of the plants, invertebrates, amphibians, reptiles, birds and small mammals in mining regions are important for establishing the extent of potential contamination.

Wildlife surveys have previously relied on a range of methods including capture and release of target species and acoustic monitoring. However, the limitations of these methods may be overcome by incorporating genetic techniques.

Wildlife naturally shed DNA into the water and soils surrounding ponds and lakes, for example in their faeces and by losing feathers, scales or fur. This environmental DNA, known as eDNA (ee dee en ay), can be analysed using powerful 'metabarcoding' techniques, in which DNA fragments from multiple species in a sample are amplified, sequenced, and then matched to an extensive database to identify the species visiting the pond.

eDNA metabarcoding has great potential to be more efficient and less invasive than traditional survey techniques, and may be able to detect species that might otherwise evade biologists. Extending beyond species detection, these tools can also be used in other areas of ecological research, such as investigating food web dynamics, interactions between predators and prey, and changes to ecological communities.

However, applying metabarcoding to eDNA samples poses limitations not found in traditional DNA barcoding studies. Traditional 'barcodes' are relatively long fragments of DNA, with a DNA sequence unique to a particular species, enabling identification. Traditional DNA barcoding is generally applied to DNA derived from tissue of an individual and not an environmental sample. In contrast, eDNA is often degraded, and the shorter fragments of DNA used for identification can result in a loss of 'taxonomic (tax-on-om-ik) resolution'. In other words, researchers may only be able to identify wildlife to the level of family or genus (jee-nuss), rather than species. Additionally, the process of amplifying DNA fragments so that they can be analysed generally favours some taxonomic groups over others, introducing a source of bias which must be accounted for.

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Dr Katy Klymus and her team at the US Geological Survey's Columbia Environmental Research Center have been evaluating the use of eDNA metabarcoding as a wildlife survey tool for ecotoxicology research. To test its effectiveness, the researchers have been sampling ponds and water tanks in mining regions near the Grand Canyon.

Local wildlife species in this area may be exposed to contaminants from historical and current mining activities. Knowing what wildlife use these water sources will aid researchers in modelling the potential biological effects of these mining contaminants. In their analysis, Dr Klymus and her team compared two DNA markers commonly used in metabarcoding to assess their ability to identify specific species. They tested these markers with water samples from man-made ponds near mine sites, as well as one sample that came from a mine containment pond.

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The team successfully demonstrated the ability of eDNA metabarcoding to detect animals that visited the water sources, though success varied between the two DNA markers they tested. Once they removed any detections that were likely due to methodological errors, a total of 18 wildlife taxonomic groups were detected in the eDNA samples.

DNA from spadefoot toads, humans, cattle and tiger salamanders dominated the samples, revealing the consistent presence of these species in the area. At one location, green sunfish were strongly detected using one of the markers; however, when using the second marker no detections were made. This illustrates the importance of selecting the appropriate marker for the taxonomic group of interest. The remainder of the animals identified by their shed DNA were detected at low levels, likely reflecting their transient usage of these water sources.

The discovery of tiger salamander and grey fox DNA in the samples is particularly notable, because these species had previously not been detected in the area using traditional wildlife survey methods. Knowing that these species are present within the mining regions ensures that researchers will include them in contaminant risk assessments and exposure models.

Unlike most frogs and toads, tiger salamanders are nocturnal and do not call, making them difficult to detect using visual and acoustic survey techniques. Dr Klymus suggests that the temporary nature of some of the water sources sampled indicate that tiger salamanders are only spending a portion of the year residing in these ponds.

Similarly, spadefoot toads were detected in abundance during the breeding season, but this species may be absent from samples collected during autumn or winter. Sampling at different times of the year may allow researchers to track the seasonal associations of species with these water sources.

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Dr Klymus and her colleagues note that the domination of DNA from a small number of abundant species, such as breeding amphibians, could mask the presence of rarer species during analysis. They suggest that this problem can be overcome by using genetic tools that block the eDNA from abundant species during the amplification stage of metabarcoding. Blocking non-target, over-abundant eDNA will improve the detection of rarer species and those that only use the water sources intermittently.



The team's research also illustrates the efficiency of the two DNA markers they tested, and the importance of selecting the appropriate markers for the taxonomic group of interest. Only one of the DNA markers used could detect green sunfish, herons, American kestrel, and grey fox DNA, while the second marker detected an additional amphibian species: the red-spotted toad.

Many metabarcoding studies have problems in identifying specific species. For example, Dr Klymus and her team could only identify some of the wildlife visiting the temporary ponds to the level of family or genus. They suggest that studies focusing on specific groups and developing study-specific databases for metabarcoding will have greater success in distinguishing between related species.

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Nevertheless, the team has successfully demonstrated the potential of these tools for complementing existing wildlife surveys. In particular, eDNA metabarcoding may help researchers detect cryptic species or those that are difficult to detect using traditional survey methods. From this research, additional species have been detected that may be exposed to contaminants in the mining regions near the Grand Canyon.

In practice, by identifying techniques to improve the accuracy and strength of eDNA metabarcoding, Dr Klymus and her colleagues have provided a basis for refining this tool for use in future wildlife surveys.

This SciPod is a summary of the paper 'Metabarcoding of Environmental DNA Samples to Explore the Use of Uranium Mine Containment Ponds as a Water Source for Wildlife', from *Diversity*, an MDPI journal. <https://doi.org/10.3390/d9040054>

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