

EVERYONE EVERYWHERE ALL AT ONCE

The emerging technology of eDNA has the potential to revolutionize our understanding of the planet's ecosystems—and our ability to protect them

By Adeline Seah and Stefan Prost

Illustration by Abby Roeser

Enveloped by a serene silence that was broken only by the distant sound of cracking ice and the occasional gust of wind, Dr. Tracie Seimon bent to collect a water sample from a crystal-clear glacial lake set in a field of snow-covered rocks up in the peaks of the Himalayan Mountains. Seimon, the director of the Wildlife Conservation Society's molecular lab, was looking for signs of wildlife. She had traveled to Sagarmatha National Park in Nepal with an international team of scientists as part of the National Geographic and Rolex Perpetual Planet Expedition. Her goal was to catalog the biodiversity of the upper Himalayan alpine ecosystem, 4,500 meters above sea level. The landscape is harsh and inhospitable, and it is difficult to imagine many animals making their home in the expanse of boulders, where avalanches are a common phenomenon. But Seimon had a secret weapon—a new technology that scientists say is key to conducting field research in remote places.

As humans and other animals move through the world, we leave behind traces of ourselves—a strand of hair, a drop of sweat, a fleck of saliva, skin, a bit of dandruff. By collecting water, soil, or air samples from an area, researchers can now detect the minute amounts of DNA in these bits of biomatter. In other words, scientists can detect the presence of animals by sampling their environment, without ever having to lay eyes on the creatures themselves. Scientists like Seimon say environmental DNA (or eDNA) technology is allowing for unprecedented opportunities to monitor and protect biodiversity. Using scat and water samples they collected from lakes and streams bordering the Khumbu Glacier, Seimon and her team were able to confirm the presence of a wide variety of organisms, including birds and mammals, among them Pallas's Cat, which was previously not known to exist in this region. The flat-faced feline had been detected in western and central Nepal, but this expedition yielded the first evidence of the species in eastern Nepal. Not only did the samples reveal the presence of the grumpy-looking cat, but DNA analysis of the scat also revealed its diet: pika and Mountain Weasel.

How does it work? The first step is to recover DNA from the sample. Running water through a filter is a common method to capture and concentrate DNA. Next, polymerase chain reaction (PCR) is performed to make many copies of a target region of DNA. These copies are sequenced to produce a string of letters (also referred to as a DNA bar code) unique to each species, much like how words encode the meaning of a sentence. The final step is bioinformatic analysis to identify which species was the source of the DNA sequenced. Depending on the interests of the researcher, the technology can be used to search for a single species or to reveal the entire biological community present in one sample. In the past decade, eDNA has been used to detect and study a wide variety of organisms and microorganisms, including insects, reptiles, fish, mammals, and even viruses.

One of the advantages of using eDNA, according to scientists, is that it makes comprehensive research in remote, demanding regions more efficient. "Performing surveys in harsh mountainous environments for biodiversity monitoring is challenging and exhausting," said Seimon. "The air is thin and can lead to altitude sickness. Bad weather can also set in, causing us to abort the survey and use our GPS devices to find our way back to our tents for shelter in whiteout snow conditions," she continued. "This is why eDNA can be such a powerful approach to enhancing survey efforts and fieldwork. From only 20 liters of water collected from 10 sites, we were able to detect well over a hundred different types of organisms across the tree of life encompassing many fungi, plants, animals, and microorganisms."

Although a vast amount of biodiversity was identified by Seimon and her team, there are limitations to the approach. "There are very few reference sequences available from Everest for comparison and species identification, so we still don't have a complete picture of wildlife," said Seimon. "Also, although eDNA helped us discover elusive species like the Pallas's Cat, there are also species that we know exist in these mountains that we didn't detect in our eDNA data, like the Snow Leopard. eDNA data should be interpreted carefully and used in combination with other approaches and methods for biodiversity monitoring."

But eDNA isn't just useful for studying wildlife in extreme environments and hard-to-reach areas like Mount Everest. Researchers are applying the technique to protect endangered species and their habitats in less remote places, such as the eastern United States, where eDNA technology is being explored for the conservation of the Critically Endangered Bog Turtle (*Glyptemys muhlenbergii*).

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The elusive Pallas's Cat can be difficult to track in its forbidding Central Asian region (right), as are tiny Bog Turtles (below) in their wooded wetland habitat.

. . . eDNA technology is being explored for the conservation of the Critically Endangered Bog Turtle.



TOP: ONDREJ PROSICKY / ALAMY STOCK PHOTO | MIKE KNOERR

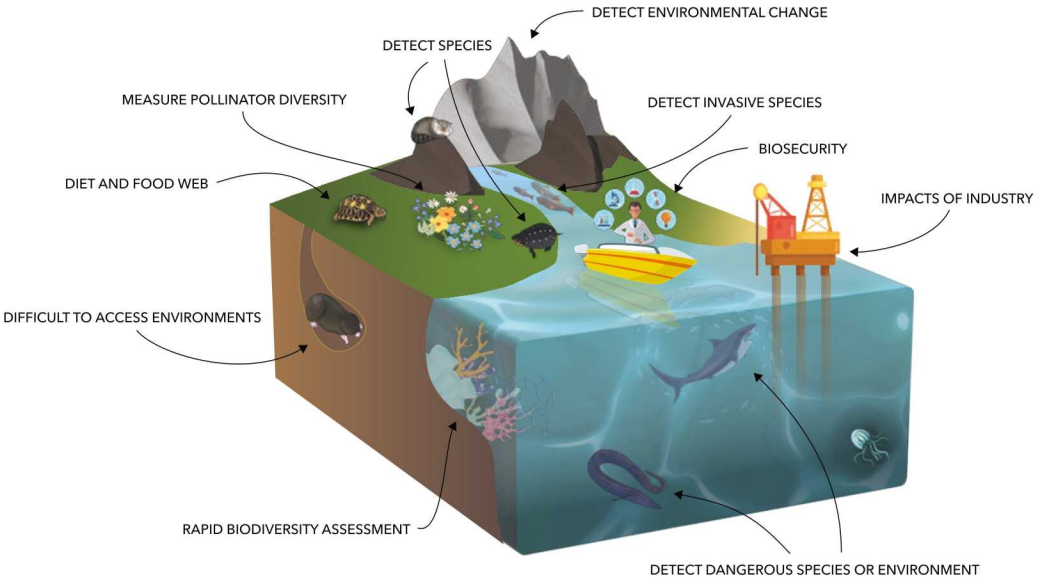
Bog Turtles are the smallest turtle species in North America and prefer to spend much of their time buried in the mud, which makes them extremely difficult to find and study. Researchers with Tangled Bank Conservation (TBC) and the Amphibian and Reptile Conservancy (ARC) have been studying how eDNA compares to camera trapping methods for detecting the presence of this shy species. "Camera trapping has proven a highly effective method for evaluating bog turtle presence or absence, abundance, activity patterns, and high use areas," said Mike Knoerr, the ARC's Southern Appalachian program coordinator. "There are situations, though, where eDNA might be more appropriate if you are mainly interested in whether the species is at a site. Camera trapping requires equipment to be left in the field, which can be stolen in highly visible sites and also requires a second trip to the site. Sometimes, due to remoteness of a site or landowner interest, a single visit is preferred."

Still, eDNA methods need improvement to perform on the level of benchmark survey methods. In a small 11-site experiment, the researchers concluded that a single camera trap left out for 21 days had a three to four times greater likelihood of detecting a Bog Turtle than a water eDNA sample collected from the same location. "We are able to detect the species at all sites where they are known to occur, but we need to further improve sensitivity and optimize our protocol," said Dr. JJ Apodaca, the founder of TBC and executive director of ARC. He is encouraged by these preliminary results and says the team will continue to optimize and validate eDNA methodology to be able to find wild populations more effectively. There are several factors that might cause a lower success rate with eDNA, such as high organic content that may interfere with DNA analysis.

Although aquatic eDNA has proven effective for monitoring wildlife, soil eDNA is still in its early stages. "In general, the more aquatic a species is, the higher the chance that eDNA is going to be successful. We have obtained good results with Alligator Snapping Turtles [*Macrochelys temminckii*] and Flattened Musk Turtles [*Sternotherus depressus*], as well as many other threatened species, including the Gopher Frog, Flatwoods Salamander, and Sicklefin Redhorse," said Apodaca. "However, it hasn't worked so well for Western Chicken Turtles [*Deirochelys reticularia miaria*] that spend much of their time on land or in the mud. It is much harder to recover DNA from soil than from water because the concentration is much lower."

Dr. Samantha Mynhardt, a scientist with the Endangered Wildlife Trust (EWT), has been having better luck with soil eDNA to reveal the presence of rare and threatened terrestrial vertebrates

POTENTIAL eDNA APPLICATIONS



ABBY ROESER







Samantha Mynhardt collecting sand samples from the entrance and area around a golden mole burrow on the South African west coast.

in South Africa over the last few years. In 2019, when she was a postdoctoral researcher at the University of Pretoria, Mynhardt partnered with the EWT's Drylands Conservation Programme to investigate the presence and distribution of two of the most elusive and threatened species of golden moles, De Winton's Golden Mole and Van Zyl's Golden Mole, on the west coast of South Africa. The eDNA investigation was based on soil collected from the linings of the subterranean burrows of golden moles across South Africa. Although the results will be formally announced once the research study has been published, Mynhardt shared that they "have made some pretty exciting discoveries using eDNA, and it has been extremely rewarding to develop this technology for the assessment of these mysterious animals." De Winton's Golden Mole was last recorded in the wild in 1937 and is listed on the IUCN Red List as Critically Endangered / Possibly Extinct.

Now with the Drylands Conservation Programme team full-time, Mynhardt is also applying the eDNA approach to the detection of other wildlife, including a collaboration with the Turtle Conservancy on threatened dwarf tortoises—the Speckled Padloper (*Chersobius signatus*) and the Karoo Padloper (*Chersobius boulengeri*)—in the Karoo semi-desert. Blending in perfectly with the isolated rocky hills (or "koppies") they preferentially inhabit, both dwarf species are as small as Bog Turtles, hide in crevices, and are seldom seen. Soil samples will be collected from sites where the Dwarf Tortoises are suspected to occur based on niche modeling. Using the DNA sequences obtained from the samples, Mynhardt will see which reptile species she can detect at each site, and especially whether Dwarf Tortoises are present at new sites. "One challenge we face is that we don't fully understand the window of detectability of tortoise eDNA in soil," she said. "So if we get a positive Dwarf Tortoise species ID in a soil sample, can we say the species is currently there? Or could it be historical eDNA in the soil? We need to know how long tortoise eDNA typically persists in the soil in order to interpret our findings accurately." Together with Jeanelize van den Heever, an undergraduate honors student at Stellenbosch University, she has just initiated a pilot study to determine the time window of detectability of tortoise eDNA in soil by measuring the accumulation and degradation of eDNA during and after exposure to tortoises in a controlled environment.

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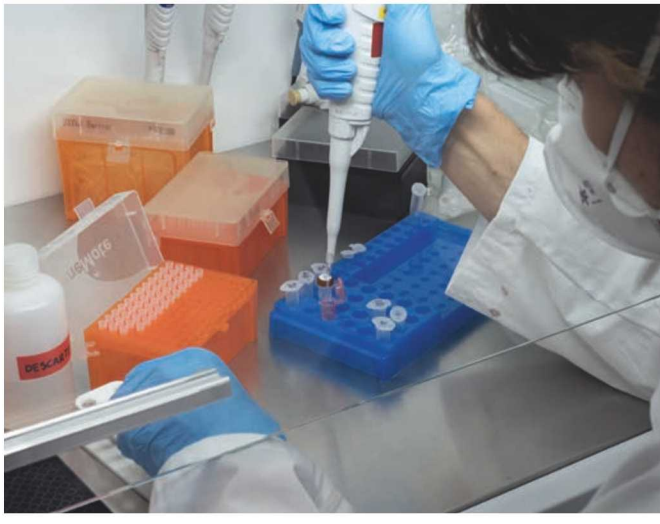
Despite its current limitations, many researchers expect that eDNA will ultimately reduce the costs of research. Over at the Smithsonian's National Zoo & Conservation Biology Institute, Dr. Tom Akre, a conservation ecologist at the Conservation Ecology Center, is part of a team that has been working on detection of the Spotted Turtle (*Clemmys guttata*) and Wood Turtle (*Glyptemys insculpta*), both of which are ranked Endangered on the IUCN Red List, from water eDNA in the eastern United States. "We are on an arc of discovery, calibration and validation," said Akre. "The benefit of eDNA, as I see it, is driving costs down. Once validated, you can use the technique for initial surveillance and bring in more expensive and/or intensive approaches if there is a hit. eDNA might be the fastest way to decide if you want to put in more surveillance time, especially for remote sites. In the future, archived samples can also be reanalyzed to look for additional species."

Beyond water and soil, researchers are now pulling DNA out of the air. Last year, two separate groups of researchers published their analysis of air samples collected from around the Copenhagen Zoo in Denmark and the Hamerton Zoo Park in the United Kingdom. Both teams were able to detect many of the zoo animals in the air DNA, including mammals, birds, and reptiles—the Danish group even identified the Guppies living in a pond exhibit. The two teams were also able to detect several wild species from the natural areas surrounding the zoos. While air sample eDNA is still in its infancy, scientists say this new frontier of research could help track animals in the wild.

Whether searching for rare and threatened species in their natural habitat, detecting pathogens that might pose a wildlife or public health concern, or surveilling for invasive species on shipping containers, scientists are constantly working to improve and find new applications for this emerging field. Scientists say that the potential impact of eDNA technology could be amplified when combined with other evolving technologies, including robotics and miniaturized genetic laboratory equipment. Already, automated eDNA samplers, such as the Monterey Bay Aquarium Research Institute's Environmental Sample Processor, have been developed to autonomously collect multiple water samples to detect protected shark species in the ocean, and pathogens and invasive fish in rivers. Smartphone-based portable technologies are being used to track and monitor







Top: Tracie Seimon collecting an eDNA sample from a high lake near Everest Base Camp.

Middle: Students in the Genomics in the Jungle course taking soil samples from animal tracks in the Los Amigos Conservation Concession.

Bottom: An instructor demonstrating how to prepare eDNA samples for DNA sequencing at the Los Amigos Biological Station.

TOP: ANTON SEIMON/NATIONAL GEOGRAPHIC | MIDDLE AND BOTTOM: THOMAS PARSONS

some of the rarest and most Critically Endangered animals in the world, the Yangtze Giant Softshell Turtle (*Rafetus swinhoei*) and the Saola, a cousin of the cow that resembles an antelope. Many of these technologies are small, cheap, sturdy, and can be powered by batteries—a striking example is the MinION DNA sequencer, which runs off a laptop and fits in the palm of your hand. A portable lab means biological samples no longer need to be sent off to local or international labs, saving time, money, and possibly permit application hurdles.

The simplicity of eDNA sample collection, combined with the affordability of portable molecular analysis technology, provides other opportunities as well. In regions with high biodiversity but little research infrastructure or funding, local scientists can use these technologies for biodiversity research and education. Nestled at the base of the Peruvian Andes, the Los Amigos Biological Station is deploying portable technologies to understand Amazonian biodiversity and to train the next generation of researchers. Over the past few years, it has hosted an innovative "Genomics in the Jungle" course that is open to everyone above the age of 18. Immersed in the wilderness, participants from all over the world learn conservation genetic techniques while building local and international synergies. Last year's course focused on the use of eDNA to track different animals in the rainforest surrounding the facility and students showed the power of the technology by analyzing mud they collected from animal tracks—and successfully identifying that they were made by a Jaguar!

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The Speckled Padloper is the smallest species of tortoise (the larger female measures less than 4 inches) and spends much of its time in small crevices, making it difficult to locate.

We are living in the age of mass extinction. Wildlife populations plunged by an average of 69 percent between 1970 and 2018, according to the WWF and Zoological Society of London's *Living Planet Report 2022*. This catastrophic rate of biodiversity loss is likely to be accelerated by climate change. Scientists hope that eDNA can help to slow down the decline in a few ways. By enabling rapid assessment and regular monitoring of ecosystems, eDNA results can potentially guide researchers on how to effectively prioritize and channel resources for conservation. The technology can also make a difference for species where regulations already exist, by showing the species is present and providing justification for the restriction or prohibition of activities that could have a negative impact. In many US states, surveillance of listed endangered species is required as part of the regulatory process before land may be developed. In principle, if a listed animal such as the Wood Turtle is present at a site, that activates a set of regulations for protection. "There are probably places where eDNA could detect Wood Turtles better than current benchmark survey methods. We would be in a better position to ensure that development doesn't harm threatened species by demonstrating the species is still there," said Akre.

But new technologies will only take us so far—to protect our natural world we also need participation from the community, involving conservationists, biologists, Indigenous groups, and anyone interested in the diversity of life on Earth. Scientists in Europe and the United States have already involved the community in large-scale eDNA surveys—in California for example, citizen scientists collect environmental samples that are analyzed by university researchers, contributing to the assessment and refinement of eDNA for biodiversity monitoring, as part of the University of California's California Environmental DNA project. We don't know how many species will eventually benefit from eDNA-based research, but broadening participation in biodiversity monitoring with the technique is something that can happen now. We need all the help we can get for wildlife and wild places everywhere.



Golden moles spend most of their lives underground, and forage in shallow subsurface burrows. The inner linings of these burrows provide an excellent source of eDNA.

OPPOSITE: NATHANAEL STANEK | RIGHT: ESTHER MATTHEW