TECHNOLOGY FEATURE

TURNING DISCARDED DNA INTO ECOLOGY GOLD

Ecologists are monitoring biodiversity using DNA shed by wildlife into the environment.

ANDREW TILKER/LEIBNIZ INST. FOR ZOO AND WILDLIFE RES.

Invertebrates such as this Borneo tiger leech (*Haemadipsa picta*) hold environmental DNA that can be used to monitor other animals.

BY SANDEEP RAVINDRAN

Kristine Bohmann visited Eswatini in 2010 with a mission: to collect bat droppings. She's not a guano enthusiast. She hoped to show that the bats were eating crop pests to convince sugar-cane farmers to preserve the bats' habitat. Usually, this would require her to scrutinize bat droppings under a microscope to find and identify insect remains. Instead, Bohmann returned to her master's programme at the University of Copenhagen with a plan. "I just came back with literally bags of bat shit and an idea."

That idea was to identify the species present in bat faeces — not microscopically, but genetically. Bohmann's studies showed that sequencing the insect DNA in bat faeces could reveal what the bats ate[1].

Ecologists are increasingly relying on DNA shed by organisms into the environment, known as environmental DNA (eDNA), for their research. Instead of trekking into the field for weeks or months to collect and taxonomically identify creatures, these scientists are tapping sources such as shed skin cells, fish scales, urine, faeces, blood and saliva for details on rare, endangered and invasive species, and to measure biodiversity.

Early eDNA surveys used the polymerase chain reaction (PCR) to amplify DNA from an individual species. But newer techniques, such as the one Bohmann used, can target a whole range of species' DNA in the same sample. And the latest methods bypass PCR altogether, instead using DNA sequencing to detect organismal signatures.

These techniques have enabled eDNA surveys to become more innovative and ambitious in their scope, opening up a whole range of taxonomic groups across large geographical regions for study. The technology still struggles when it comes to estimating population abundance, and requires expensive lab equipment and sophisticated bioinformatics skills. And for all eDNA's reliance on twenty-first-century technology, ecologists still need to put in the hard yards outdoors to collect the samples. But as eDNA surveys become cheaper and more accessible, it's increasingly becoming a powerful complement to conventional field-biology techniques.

Ecologists could certainly use the help. "Biodiversity is hugely threatened all around the globe and we don't have the amount of experts actually even to document what we have now," says Philip Francis Thomsen, a molecular ecologist at Aarhus University, Denmark. "We need to describe what we have while we still have it, and environmental DNA could be one way."

LESS LIFTING, MORE FILTERING

A big part of ecology and conservation biology is knowing what species are where. To survey reclusive salamanders known as ▶

hellbenders (*Cryptobranchus alleganiensis*) in the southeastern United States, wildlife ecologist Stephen Spear had to snorkel or walk through creeks, lifting large rocks and peering underneath. He could survey at most two or three nearby sites in a day. Yet eDNA means he can survey many more — he just needs to collect a litre of water from different points along rivers and streams. "If they're eDNA-positive, maybe that's where you focus on going more in depth," says Spear, director of wildlife ecology at The Wilds, a non-profit safari park and conservation centre in Cumberland, Ohio.

Leaving stones unturned reduces physical risks, as well. "I've had rocks dropped on my arm, and I've had colleagues who've actually broken bones from rocks inadvertently dropping," says Spear.

And it's not only the ecologists who benefit — eDNA "is quite good for biodiversity because we're not having to kill anything to actually study it", says Mark de Bruyn, an evolutionary biologist at the University of Sydney, Australia. De Bruyn travelled across southeast Asia for six months during his PhD collecting tiny pieces of freshwater prawns. Now, his PhD student Alice Evans at Bangor University, UK, has only to collect water samples. Whereas de Bruyn needed a week or more at each location, for Evans' project, she was done in a day or two, spending less than a month overall in the field.

FROM ONE SPECIES TO MANY

Using PCR to amplify species-specific DNA is a relatively easy way to monitor individual invasive or endangered species. But newer techniques enable ecologists such as Evans to get data from many different taxa at once — in her case, from mammals, fish, crustaceans and amphibians. Back in 2010, Bohmann was faced with the problem of needing to identify dozens of insect species from the same samples of bat guano. Fortuitously, her adviser, Thomas Gilbert at the University of Copenhagen, had just developed a technique that could help.

In eDNA 'metabarcoding', DNA is amplified by PCR using short segments of DNA, called primers, that have a unique tag at one end and that target genome sequences common to organisms across an entire taxonomic group. In Bohmann's case, she used universal primers to amplify and sequence insect eDNA from more than 100 faecal samples in parallel, which were then differentiated on the basis of their unique tags.

Such data can provide a broad overview of a region's biodiversity. "Environmental DNA catches more species, in general, than do experts going out [into the field]," says Thomsen. His metabarcoding surveys have detailed fish communities off the coasts of Denmark and Greenland, as well as population-level genetic data from a gathering of whale sharks (*Rhincodon typus*) near Qatar. "I don't know of any other method that is so broad that you can actually get information from across the taxonomic tree of life, all the way from bacteria to whales," says Thomsen.

Surveys of eDNA can also be cheaper than conventional methods, says Kat Bruce, the co-founder and managing director of NatureMetrics, which provides eDNA analysis services from its base in Egham, UK. A small survey near a wind farm in the North Sea involving three water samples cost £600 (US$750) and identified two-thirds of the fish species that were recorded by a two-year, £150,000 monitoring programme, she says.

Plus, anyone can collect eDNA samples, making it useful for citizen-science projects. In a UK survey of the endangered great crested newt (*Triturus cristatus*), eDNA analyses of pondwater samples collected by members of the public correctly identified 219 of 239 sites as supporting newts[2]. Given that the volunteers had received no training, the researchers suggest that even minimal training could reduce the false-negative rate.

Researchers are setting up similar projects with ecotourism companies in the Amazon. Tourists would collect eDNA samples while visiting national parks, thus contributing to long-term biodiversity monitoring. "What you're doing is you're taking away the bottleneck of needing expert observers, and then you can study much larger areas," says Douglas Yu, a molecular ecologist at the University of East Anglia in Norwich, UK.

> ***"We were scared out of our minds; we thought we'd die horribly, but in a good cause."***

CREATIVE SAMPLING

And you can study geographical regions using an ever-widening array of sample sources, from flowers and leeches to air. "The imagination is setting the limits to what sample types you can detect traces of animals and plants in," says Bohmann.

In a study published this year, Thomsen extracted eDNA from wild flowers picked from two grassland sites in Denmark and detected more than 100 species of insects and other arthropods that had visited them, including pollinators, predators and parasites[3]. Previously, he would have had to spend all day planted next to a flower, watching and identifying the insect species that interacted with it. Yu and his colleagues once followed a brown bear (*Ursus arctos*) catching salmon for her two cubs in Alaska, hoping to collect bear eDNA. When the bear had finished munching on a fish, they hurriedly grabbed the saliva-slathered leftovers and swabbed them.

"We were scared out of our minds; we thought we'd die horribly, but in a good cause," says Yu. Luckily, the researchers were unharmed — and in possession of fresh bear DNA. "We could use the eDNA to actually identify individual bears, and then you can start doing [animal] counts from that," he says.

Yu and others have also relied on DNA collected by invertebrates — from blood-sucking mosquitoes and flies that feed on decaying animal carcasses or faeces to marine sponges that filter eDNA from seawater. To survey a remote Chinese nature reserve nearly the size of Singapore, Yu asked park rangers to collect leeches as they walked trails during the rainy season in 2016. Some 160 rangers rounded up about 30,000 leeches, which contained preserved eDNA from salamanders, frogs, mammals and birds. "Leeches seem to be like the equivalent of –80 °C freezers in the tropics," he says.

SEQUENCING CHALLENGES

The utility of eDNA notwithstanding, wildlife ecologists needn't fear for their jobs, says Thomsen. For one, taxonomic specialists are still required to build the reference databases against which eDNA is matched to identify species. "At the moment, only about 15% of the Amazonian fish species that we find can be given a name," says Bruce.

Surveys of eDNA also pale in comparison to conventional ecological techniques for estimating population sizes, such as mark and recapture, in which individuals in a population are captured, marked and released, followed by another later capture when the number of marked organisms is counted. In quantitative PCR (qPCR) and metabarcoding approaches, the amplified DNA doesn't necessarily reflect its abundance in the environment, making it difficult to translate a PCR result into population estimates. And DNA-sequencing-based strategies require good reference databases, not to mention particularly comprehensive sequencing, because eDNA samples are chock-full of microbial DNA.

Contamination is perhaps the biggest challenge when dealing with trace amounts of DNA, says Spear. Researchers have to be meticulous about changing gloves, boots or wetsuits between field sites. They need separate lab facilities for eDNA extraction and PCR amplification, and clean spaces where they can handle the DNA while wearing face masks and suits.

Commercial eDNA labs can help. José Luis Mena and Hiromi Yagui, ecologists with the conservation group WWF in Peru, worked with biotechnology company Spygen in Le Bourget-du-Lac, France, on a pilot eDNA project in 2015 to survey mammals in Peru's southwestern Amazon. The scientists have since been conducting a large-scale survey of freshwater vertebrates in the northwest Amazon in Peru with the help of NatureMetrics.

"We send them the samples, and they give us the results — a list of the species and their numbers," says Yagui. Samples of eDNA turned out to be particularly useful for detecting species such as manatees, which are difficult to identify through other field methods, she says.

The researchers used several other

established technologies, including sonar and camera traps, but eDNA was new to them. "At the beginning we had some scepticism about its utility for our questions, but the first results were fantastic, especially because of eDNA's complementarity to the other techniques," says Mena. But at about £200 per sample, the process isn't cheap. And researchers also have to wait patiently for the results, which can take weeks to come back and even longer for more complicated analyses.

NO LAB, NO PROBLEM

Using newer tools, researchers can increasingly get immediate results in the field. For his hellbender eDNA surveys, Spear used the two3 — a smartphone-based portable qPCR machine from Biomeme in Philadelphia, Pennsylvania. Whereas a typical qPCR machine can run 96 samples at a time, the two3 can run only 3 (its successor, the Franklin range of machines, can run a maximum of 9). But instead of waiting days to receive the results from the lab, the two3 delivered them in minutes, and with comparable accuracy. "This sort of system could be really useful if you need to know if a species is there very quickly," says Spear.

But using portable instruments in the field can be a challenge. Joseph Russell, a microbial genomicist at MRI Global, a non-profit contract-research organization in Kansas City, Missouri, used a portable sequencer for monitoring viruses transmitted by arthropods such as mosquitoes and ticks in the Everglades National Park, Florida, and says it was "really logistically difficult and stressful". Not only did the wind and conditions scatter their sample tubes and reagents, but sequencing for even a few hours completely drained their laptop battery.

As a result, Russell created the suitcase-sized Mercury Lab, a portable lab that contains a workbench, cooler, centrifuge, integrated computer and enough power to comfortably run portable qPCR and sequencing experiments in remote field sites for weeks at a time. "Rather than coming back with multiple coolers full of samples, if you could just come back with a thumb drive full of data it would make a lot of things easier," says Russell.

That's a far cry from what Bohmann expected when her bat eDNA study was published in 2011. "I thought, nobody's going to be interested in this," she says. "I didn't know I had come into a brand new field." ■

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2. Biggs, J. *et al. Biol. Conserv.* **183**, 19–28 (2015).
3. Thomsen, P. F. & Sigsgaard, E. E. *Ecol. Evol.* **9**, 1665–1679 (2019).

VED CHIRAYATH, NASA AMES LAB. FOR ADVANCED SENSING

A drone is used to take photographs below the surface of the water.

Drone takes to the skies to image offshore reefs

Scientists are using uncrewed aircraft to map the topography of Guam's coral reefs.

BY ANDREW SILVER

Researchers from NASA and the University of Guam have remotely mapped a large stretch of coral off the coast of the western Pacific island.

In May, the research team took less than two weeks to study two marine habitats using an uncrewed aerial vehicle (UAV), or drone, to create a centimetre-resolution digital model of the reef structure. Previously, the survey, which could help conservation efforts, would have taken divers a month. The team hopes that its efforts will help researchers to better track changes in reef structure over time.

"You'd be able to get so much coverage in a small amount of time," says one of the project's principal investigators, Romina King, an environmental geographer at the University of Guam in Mangilao, a village on the eastern shore of the island.

About one-third of corals in the shallow waters around the US territory have already died following bleaching events between 2013 and 2017, when warm temperatures caused corals to expel the important algae that give the coral their colour and provide them with essential nutrients, says King. Scientists lacked detailed measurements of reef structure, so there was no baseline to identify areas where conservation efforts were and weren't working. Now, thanks to drones, that's changing.

UAVs are popular with hobbyists, and increasingly, says King, among Earth scientists. In May, meteorologists in the United States began launching drones to study intense rotating thunderstorms called supercells across the Great Plains.

The Guam team's UAV is a US$15,000, 6-rotor carbon-fibre drone made by technology company DJI, based in Shenzhen, China. The Matrice 600 is outfitted with GPS sensors, hard drives, a memory card, a $90,000 RGB 'fluid cam' that corrects the distortion caused by the surface of the water to photograph beneath the waves, and a 7-colour ultraviolet sensor for testing NASA coral-identification technology. Including batteries, the assembly weighs about 12 kilograms.

The team sent the UAV on short hops from the shore to pre-set coordinates 30.5 metres above Guam's Tumon Bay and Tepungan Bay reefs. In total, the researchers collected about 11 terabytes of data across roughly 5 square kilometres, including image files and flight parameters such as speed, altitude and spatial orientation. NASA is using a supercomputer to process and stitch those data into 3D models of the reefs — a process that could take six months.

Ved Chirayath, director of the NASA Ames Laboratory for Advanced Sensing in Mountain View, California, developed some of the UAV's imaging technologies, and says he chose the Matrice 600 for its ruggedness: if one of the six batteries or rotors dies, it can still fly. Still, when the power levels of two batteries unexpectedly dropped mid-flight and the drone had to make an emergency landing in shallow water, the team had to ship in a backup from California to complete its work.

"Field work is hard, UAVs fail, instruments die," Chirayath says. And then there's the human element: "There's nothing that makes [you] more seasick than staring at a drone screen on boat." ■

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