Environmental DNA helps researchers track pythons and other stealthy creatures

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It's no secret that Florida has a snake problem. The Burmese python, which can reach up to 200 pounds and stretch to more than 20 feet, first became common in the Everglades in the late 1990s, likely as escaped pets. The snake quickly settled into its new home, breeding and taking down rabbits, bobcats, and other native animals in its path.

Wildlife managers in Florida turned to expert snake hunters, electronic tracking devices, and search dogs to wrangle the pet-turned-ecosystem-wrecker and had little success. The snakes, although massive, are hard to find in the south Florida habitat. "They're well camouflaged, secretive, and often slow moving," says geneticist Margaret Hunter of the US Geological Survey, Wetland and Aquatic Research Center.

Biologists thought the Arthur R. Marshall Loxahatchee National Wildlife Refuge was one place that was safe. Pythons have on rare occasion been spotted there, but only in areas easily accessible to humans, suggesting that the snakes were likely released pets rather than a growing population. But from 2014 through 2016, Hunter combed the waters in and around the refuge for environmental DNA (eDNA)—the trail of DNA left behind by an organism in sources such as feces, mucus, gametes, and shed skin or hair. The results suggested that the python's DNA was, in fact, widespread throughout the refuge.

Hunter is one of a growing group of researchers who are using eDNA to track invasive species that they'd like to remove and vulnerable species they'd like to protect. Hunter also developed an eDNA test for manatees that's more sensitive than traditional aerial surveys.

But challenges remain before eDNA can become a widely used tool for conservation biology. The technique carries the risk of false negatives and false positives. And it's not always clear how to make eDNA results translate into putting eyes, and hands, on the actual animals that managers are after.

Burmese pythons are well camouflaged in the Everglades, but the trail of DNA they leave behind helps researchers track their presence. Image courtesy of USGS/Emma Hanslowe.

A Genetic Trail

Researchers recover eDNA by first developing primers that allow them to amplify the genetic material left behind in environmental samples via a version of the polymerase chain reaction technique. To track a single species, researchers use primers that amplify a portion of DNA unique to that species (1). Those surveying multiple species or even an entire community of organisms select more generic primers and sequence all of the diverse strands by using high-throughput sequencing (2).

The concept of extracting DNA from environmental samples to identify the presence of organisms stems from work in the late 1980s. Microbiologist Gary Sayler of the University of Tennessee and colleagues described the technique in an article detailing the extraction of microbial DNA from sediments (3). Because microbiologists couldn't culture many bacterial

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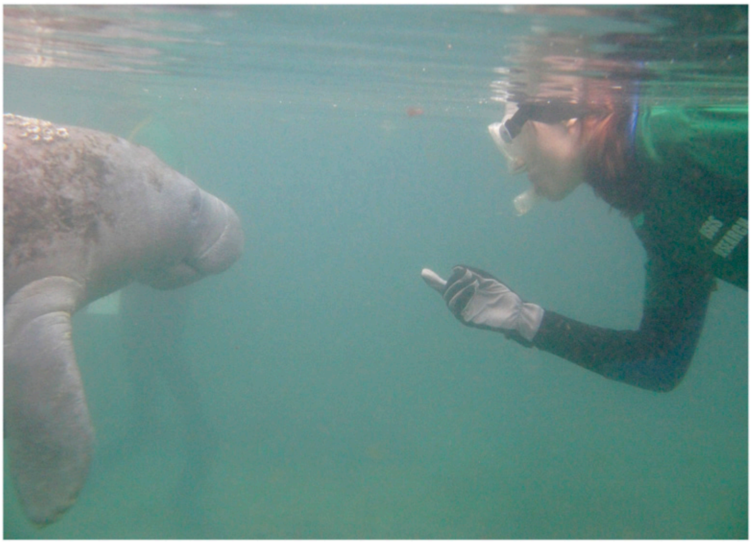

Margaret Hunter comes face to face with a manatee, one of several threatened species that researchers have tracked with eDNA. Image courtesy of USGS/Gaia Meigs-Friend.

species in the lab, eDNA offered a broader view of microbial diversity. In 2003, evolutionary geneticist Eske Willerslev of the University of Copenhagen and colleagues applied the concept to the retrieval of animal and plant DNA from environmental samples (4). They focused largely on using ancient DNA preserved in permafrost and cave sediments as a record of animal and plant diversity. About a decade ago, conservation biologists began recognizing the potential for eDNA to track animals that were otherwise difficult to survey because they were small, elusive, hard to identify, or easily damaged by traps or other physical survey methods (2).

In 2008, in one of the first conservation-related uses of eDNA, zoologist Gentile Francesco Ficetola and colleagues reported using DNA sampled from pond water in France to confirm the presence of the invasive American bullfrog (5). In this proof-of-concept study, Ficetola, now of the University of Milan, detected bullfrog eDNA only in sites known to have bullfrogs. Others on the team later showed that the technique is more sensitive to bullfrog detection than traditional monitoring methods (6). Since Ficetola's study, conservation biologists have tracked a wide range of species using DNA extracted from soil, fresh water, salt water, and even air.

Collecting the samples for eDNA analysis is straightforward. Hunter sends team members into the Everglades and surrounding areas on airboats. At each sampling site, with gloved hands they dip three to five 1-liter sterilized Nalgene bottles into murky water. John Butterfield, a former technician in Hunter's lab, recalls riding past many alligators on his way to collect samples. "You would hear them out there grunting," he says. But in about 40 hours of sampling, he never saw a python.

Python Problems

In fact, the snakes are extremely well camouflaged even for experienced Everglades python hunters. "Our detection probability is a limiting factor in our efforts to control them," says Tylan Dean, Biological Resources Branch Chief of Everglades National Park and Dry Tortugas National Park.

Managers remove 100–200 snakes per year from Everglades National Park, but the python population continues to grow with a distribution now spanning southern Florida from coast to coast. Searching for another detection tool, Dean has worked with a handful of researchers, including Hunter, who are looking for ways to turn eDNA sampling into an effective means of locating pythons.

In 2013, Hunter sampled water from python enclosures in the laboratory and then from regions in and around Everglades National Park where pythons had previously been sighted. She detected python DNA in all of the laboratory water samples and in 17 of the 21 Everglades sites (7). "If [python eDNA] is there, we are able to detect it 90% of the time," she says.

Hunter then took her detection tool to where python presence was far less certain. From 2014 through 2016, Hunter's team repeatedly sampled in and around the Loxahatchee National Wildlife Refuge. In fall 2014, she got her first python DNA hit. Managers were skeptical. "The refuge has a lot of visitors and managers, and no one had seen a snake in places you would expect to see them," says Hunter. Then, on a summer evening in 2015, someone saw what appeared to be an adolescent python moving across the refuge parking lot. And in fall 2016, a US Fish and Wildlife Service law enforcement officer accidentally drove over a 10-foot python on a levee along the refuge's perimeter. With eDNA, it's helpful to have that confirmation of a true detection, says Hunter.

Protecting Threatened Species

Applications for eDNA go beyond hunting unwanted invasive species. The strategy has the potential to help researchers identify the geographic ranges of vulnerable species. Hunter and her team also designed primers for amplifying a region of manatee DNA from water samples (8). Earlier this year, in a proof-of-concept study, they reported that this eDNA test for three species of manatee offered a higher probability of detecting the animals than did traditional aerial surveys (8).

Meanwhile, marine ecologist and population geneticist Stefano Mariani of the University of Salford in the United Kingdom and his team have developed eDNA tests for sharks. These elusive animals, many of them threatened, are difficult to survey by using traditional methods, says Mariani. "They have large ranges," he explains. "They are not easy to capture without distressing them or incurring some possible physical trauma for people involved." Mariani's team used eDNA to identify the presence of more than 20 shark species in the Caribbean and the Coral Sea (9). In May 2018, he and colleagues reported the results of a direct head-to-head test of the effectiveness of underwater visual censuses and baited videos versus eDNA (10). "We had more than twice the chance of hitting any shark in our sample than the other methods and almost twice as many species on average than with the other methods," he says.

Translating Data into Action

Collecting eDNA is so straightforward that citizen researchers could easily take part. Mariani notes that many people sailing around the world for leisure could sample water along their routes for researchers. By noting the location of each sample using mobile phone geolocation, they could map the distribution of species. "That could upscale dramatically the amount of information you could get from all over the sea," says Mariani. Indeed, in New York state, elementary, middle, and high school students are already collecting water samples for eDNA to help researchers at Cornell University track endangered and invasive fish species (11).

But interpreting eDNA results can be tricky. Tiny amounts of cross contamination in the field and lab could result in positive detections where animals aren't present. "You can get these low signals that are either critically important or not reflecting the truth," says ecologist Caren Goldberg of Washington State University, whose team has developed eDNA tests to monitor for a wide range of amphibians, including the endangered Sierra Nevada yellow-legged frog. In those cases, the eDNA is there, Goldberg says, but the interpretation of what that means can be wrong. Goldberg, for example, cannot control for moose that carry water in their coats from one pond to another, potentially transferring DNA of fish and other species.

eDNA studies may also miss species. When primers didn't amplify DNA samples as intended, Mariani failed to detect nurse sharks in the Caribbean, even though his team witnessed some while sampling (9). Assuming working primers, Hunter notes that there are models to help account for false negatives. But models that filter out false positives are still under development—researchers still need to figure out, for example, how many samples they need to craft such models and how to interpret detections that occur initially but not from a later sampling.

Then there is the challenge of translating results into management action and practices. eDNA may signal that a species is present, but it usually cannot indicate abundance in open water systems. And for many conservation goals, researchers still need to see or physically capture the animals they're managing—a big challenge in cases such as the python. Rolf Olson, refuge manager at the Loxahatchee National Wildlife Refuge, would like to know more about eDNA and the samples' origins. "Was it recent?" he asks. "Could it have come in from some other place?" Hunter has conducted some preliminary tests of how long eDNA stays in the environment, although results could vary greatly across different microenvironments.

"You can get these low signals that are either critically important or not reflecting the truth."
—Caren Goldberg

After Hunter's tests came back positive for python, Olson says his team significantly increased the number of volunteer hours the refuge devotes to visually monitoring for invasive reptiles. The volunteers have never seen a python. And as far as Olson knows, that suspected python spotted in the parking lot is still at large.

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