Untouchable Methods, Unparalleled Data

NONINVASIVE TECHNIQUES YIELD NEW INSIGHTS INTO WILDLIFE

By Dana Kobilinsky

About 15 years ago, James Derr was studying American bison on the North Rim of the Grand Canyon. A rancher named Charles “Buffalo” Jones established a herd of cattle-bison hybrids there in the early 1900s for a commercial beefalo herd, but after a few years, Jones’ investors pulled out. The operation folded, but the hybrids remained.

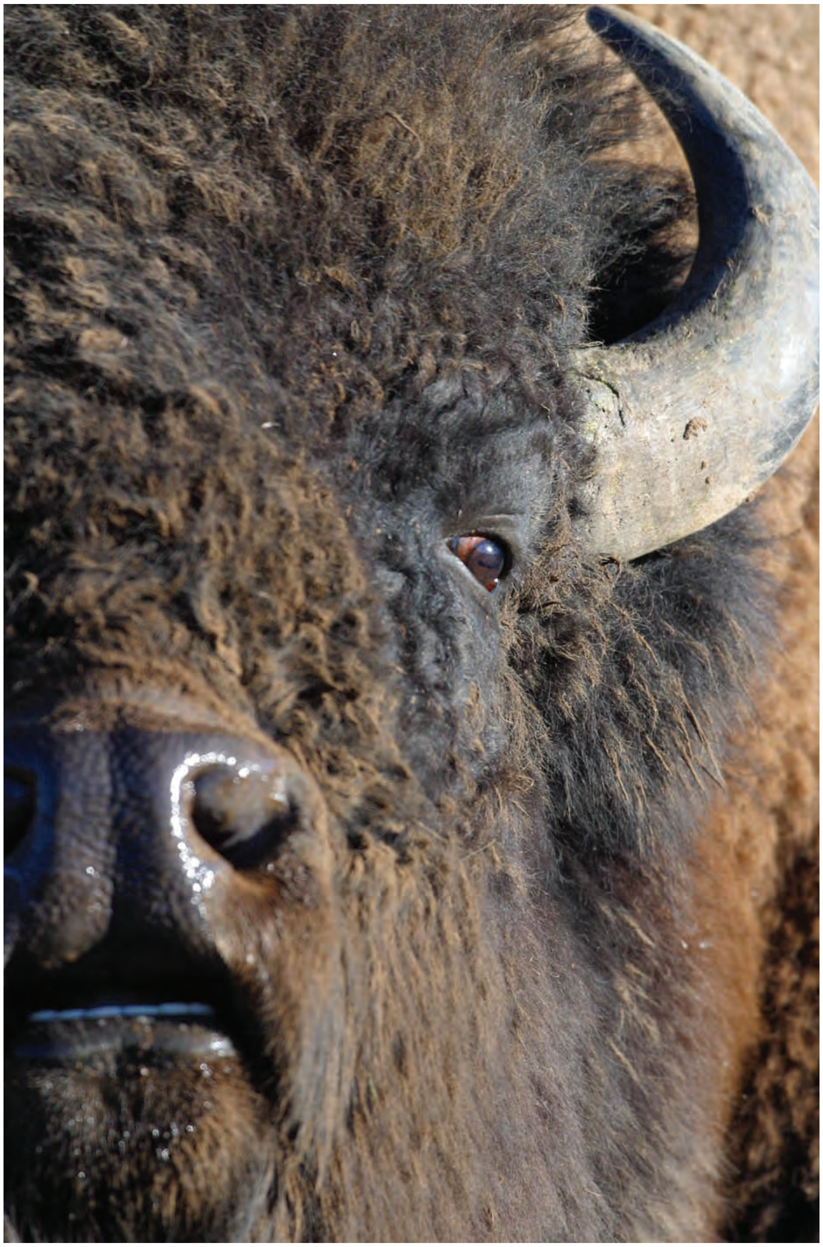
Bison graze in an open meadow on the North Rim of Grand Canyon National Park. Scientists used DNA samples from bison hair to learn more about their genetics.

Credit: National Park Service

Derr wondered how much cattle DNA still remained in today's herd. The National Park Service was working to reduce the herd, which was muscling out deer and elk. The InterTribal Buffalo Council, a collection of Native American Tribes looking to restore bison to native land, wanted some of the animals to boost other herds. Understanding their genetics could help managers deal with them, Derr thought.

But catching the bison (*Bison bison*) to collect DNA samples proved difficult. Derr and his colleagues tried to dart them with anesthesia to gather blood samples for genetic testing, but the bison kept evading them. They deployed airplane pilots to radio scientists on the ground where the bison were. They tracked hoofprints across the Arizona landscape, but when they got within 200 yards of the animals, the bison disappeared into the distance.

▼ **James Derr has spent much of his career using a variety of techniques to study bison genetics.**



Credit: Diane Hargreaves

"We spent four or five days chasing bison," said Derr, now a professor in the School of Veterinary Medicine at Texas A&M University.

Almost giving up hope, Derr noticed something. Before the bison vanished from the horizon, they jumped over logs and brushed against trees, leaving clumps of hair behind. Instead of drawing blood from tranquilized bison, he realized, his team could test those hair samples. The method worked. While Derr and his team did find some cattle DNA, they found the animals were mostly bison, clearing the way to relocate 124 of them and reduce the herd.

Noninvasive techniques like this are giving researchers information that would have been difficult or impossible to collect otherwise. Trail cameras clue in researchers to which animals are present in an area, even if they're too elusive to be seen. Drones provide an overhead view and leverage other technologies to better detect wildlife. Acoustic monitoring captures the sounds of an ecosystem for researchers to decipher. Environmental DNA can tell scientists what species occur in an entire aquatic ecosystem just from a water sample from a stream.

These types of techniques are growing in popularity for a host of reasons. In some cases, it's due to concerns about animal welfare and safety, particularly in handling endangered or threatened species. But it's also due to the tremendous information these methods can provide. As technologies improve, they are helping scientists develop vast datasets that would have been impossible to achieve otherwise.

Of course, researchers still often need trapping, netting, darting and handling to gather the information they're looking for. But sometimes, noninvasive techniques provide unparalleled data without ever touching a live animal.

Credit: Frank Chapman, courtesy American Museum of National History

Credit: Frank Chapman, courtesy American Museum of National History

In the 1890s, Frank Chapman became the first researcher known to use camera traps for science. In the image to the left, he captured a puma striding across Barro Colorado Island in Panama's Canal Zone. The image above shows his set up. Chapman used a banana as bait and strung a trip wire so that when an animal approached, it triggered an explosion of magnesium powder that lit up the night and clicked the shutter of a camera mounted on a tripod nearby.

Caught on camera

The idea of using noninvasive techniques isn't new—some techniques have been used for a century. Frank Chapman, curator of birds at the American Museum of Natural History, is considered the first researcher to use camera traps for science. While many of his colleagues used rifles to collect and study wild animals, Chapman used a lens. "We want a census of the living, not a record of the dead," he wrote in National Geographic in 1927.

Studying elusive wildlife on Barro Colorado Island in Panama's Canal Zone, Chapman borrowed a technique from George Shiras III, a U.S. congressman who pioneered camera trapping for personal use in the 1890s. Using bait, Chapman strung a trip wire so that when a passing cougar (*Puma concolor*) or Baird's tapir (*Tapirus bairdii*) approached, it triggered an explosion of magnesium powder that lit up the night and clicked the shutter of a camera mounted on a tripod nearby. He even caught on camera a white-lipped peccary (*Tayassu pecari*), providing evidence they had been there before going extinct.

Today, researchers use camera traps for similar reasons but with more sophisticated equipment. Motion sensors trip the shutter. Infrared and night vision technology allow cameras to capture nocturnal scenes without scaring off the animals. Cellular connections allow researchers to see images in real time. Memory cards can hold thousands of pictures, which artificial intelligence can decipher later.

"Around 2008, we finally got a decent digital camera trap," said TWS member Roland Kays, a professor at North Carolina State University and the lab head at the North Carolina Museum of Natural Sciences. That was a few years after a U.S.

Credit: Matt Jones

▲ Roland Kays sets a camera trap in the forest of North Carolina. Camera trapping is one noninvasive method of collecting data on wildlife species.

Forest Service technical report, "American marten, fisher, lynx, and wolverine: survey methods for their detection," came out, providing guidance on a range of techniques to biologists for studying carnivores (Zielinski and Kucera 1995).

The paper was a "bible" for many biologists, said Kays, who has worked on projects like Wildlife Insights, which provides a global database of camera trap photos that other researchers can use, and Snapshot USA, a collaborative project that collects mammal survey data through camera traps in all 50 states.

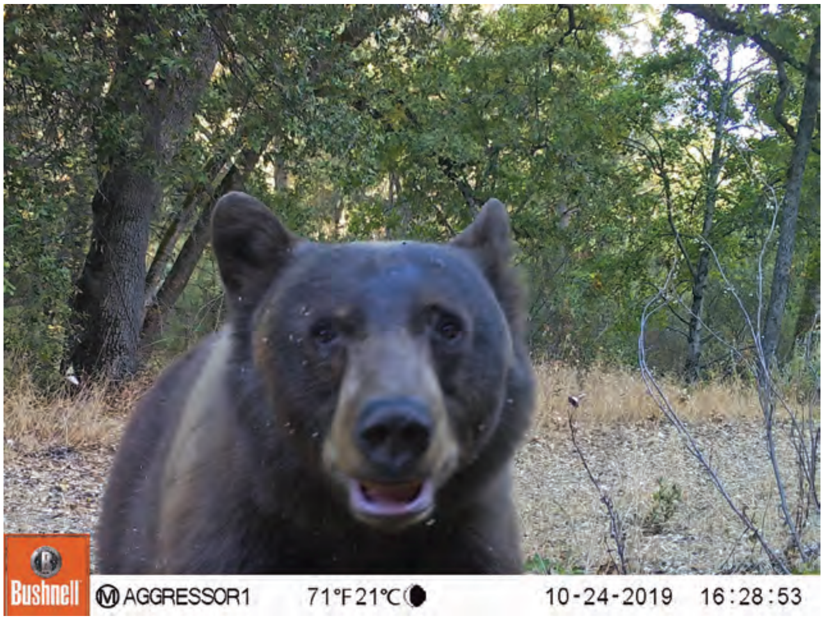
Looking at images on a new memory card is like "Christmas morning," he said. Going through photos from camera traps around Albany, New York, Kays spotted a fisher (*Pekania pennanti*)—the first one ever recorded in the area in 2009. "That was one picture that totally changed the course of my research," he said.

For Kays, camera trap studies on their own can yield important insights on elusive animals. "There's a lot of research questions you can only answer from these pictures," he said. Combining them into large-scale projects can produce a whole new level of data, though, and spot global trends that may otherwise be hard to pinpoint.

The pace of the change on the planet is so fast that researchers constantly need wildlife data to track how species are doing, Kays said. "Noninvasive techniques are a part of that solution."

Seeing the unseen

Other techniques, like environmental DNA—or eDNA—have also been used to help researchers make surprising findings. By taking samples



Credit: Snapshot USA

◀ This black bear (*Ursus americanus*) camera trap photo was included in Snapshot USA. Roland Kays created the collaborative project to collect mammal survey data from camera traps throughout the U.S.

gathered from the water—or sometimes even the ground or the air—researchers can find traces of DNA from species that are present in the environment, even if no one ever sees them.

When Krista Ruppert, a PhD student at Mississippi State University, uses eDNA to study amphibians, she places a filter into a water body that captures the DNA. Then, she places the filter in a tube with a preservation medium and takes it back to the lab. There, she extracts the DNA from the filter and uses PCR, or polymerase chain reaction, which amplifies the DNA from the target species she's looking for.

When Ruppert compared eDNA with trapping data in water bodies with known populations of sirens (*Sirenidae* spp.)—a type of aquatic salamander—she found eDNA gave her a 98% positive detection rate. She even detected sirens far west of their known range in places where they hadn't been detected since 1880 (Ruppert et al. 2022).

Using eDNA has even helped her conduct research on private lands she might not have been able to access otherwise. "People are more likely to help if you just want to take a little water and aren't disturbing things or spending a lot of time there," she said.

Sometimes, the public can even get involved in data collection. In British Columbia, Mark Louie Lopez, a Liber Ero and iTrackDNA postdoctoral fellow at the University of Victoria, is working with Indigenous people to collect samples from lakes and rivers. By tapping into their knowledge

▼ **Researchers Iain Robertson, Bill McKnight and Matthew Watkins collect eDNA samples to identify beetle presence in Oregon. Environmental DNA allows researchers to learn about species within an ecosystem from just a sample of water, sediment or even air.**

Credit: Molly McKnight

about what species they are likely to find, he can narrow down what DNA to look for.

"The idea is, eDNA technology will help you see the unseen," Lopez said.

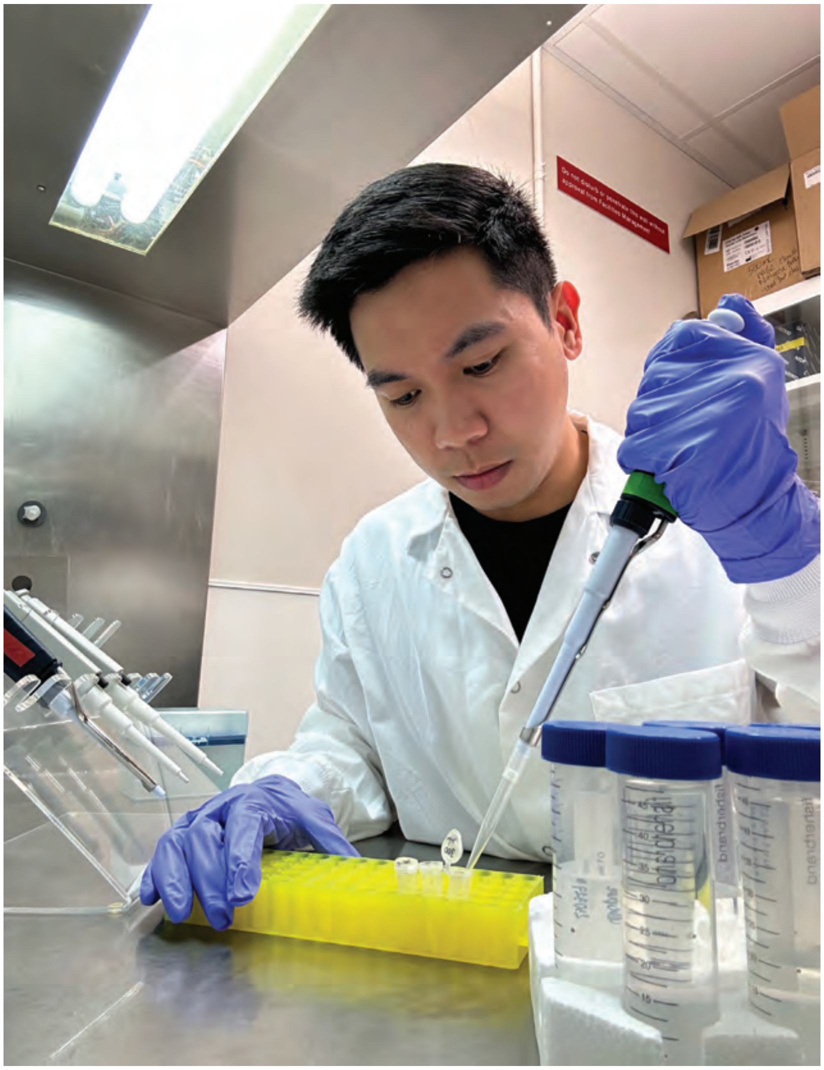
In one case, he collected sediment layers from a lake bottom and used stable isotope analysis to date the layers as far back as 1905. Indigenous communities spoke of a fish species—the lake whitefish (*Coregonus clupeaformis*)—that was present before 1950. "Then, suddenly, it was gone," he said. "Our DNA detection aligned with the Indigenous stories."

Lopez has also used eDNA to confirm snow tracks of different species, another noninvasive technique. Since 1995, the Wisconsin Department of Natural Resources' Carnivore Tracking Program has also used this tracking technique by soliciting the help of locals to monitor gray wolves (*Canis lupus*) through paw prints in the snow.

"They would train volunteers—citizen scientists—on tracking skills and data collection methods when it snowed in mid-November through the end of March," said Annie McDonnell, the wolf monitoring and tracking coordinator with the Wisconsin DNR. With this information, the DNR was able to estimate the number of individuals within a pack and understand their territories.

Independent biologists Laura Matthias and Carrina Maslovat have used eDNA to search for cryptic sharp-tailed snakes (*Contia tenuis*) in

▼ Mark Louie Lopez extracts suspended eDNA from filters.



Courtesy Mark Louie Lopez

▶ The Wisconsin Department of Natural Resources' Carnivore Tracking Program trains volunteers to identify wolf footprints in the snow.

Credit: Wisconsin Department of Natural Resources

Credit: J.F. Dwyer

▲ **A drone helps researchers inspect an osprey (*Pandion haliaetus*) nest to identify entanglement hazards.**

southwestern British Columbia. Listed as endangered in Canada, the rare snake is hard to detect using traditional methods. Gathering material beneath artificial cover objects (Matthias and Maslovat 2020), the two found that eDNA could detect sharp-tailed snakes in places they were never known to exist. Since then, they've honed the technique to find the snakes in the natural environment, too. "This demonstrates that the tool is working and can lead to conservation successes," Matthias said.

Tech takes flight

In the last decade, drones have taken off for wildlife research. Sometimes, they simply give a bird's eye view. Other times, they're much more sophisticated, like using multispectral or thermal imagery to detect species most ground or other aerial surveys would never find.

Drones have been used to help researchers survey hard-to-reach cliffs in Hawaii to rediscover plants thought to be extinct. They have also aided in detecting ground nesting birds like Canada geese (*Branta canadensis*) to conduct egg oiling that reduces their reproductive output. That benefits species like desert tortoises (*Gopherus agassizii*), since the geese destroy their habitat.

Drones are safer for biologists to fly than helicopters, and they're generally less disruptive to animals, but they're not without impact. Different animals react to drones in different ways, said Rick Spaulding, the chair of TWS' Drone Working Group and senior biologist with the consulting firm ManTech Advanced Systems International. Researchers are working to reduce the drones' impact by varying the distance between the drone and the subject wildlife.

"As with any animal, there's a spectrum. They react differently," Spaulding said. "Some are sensitive, and some don't care."

When drones first got underway in wildlife work, biologists often feared it would put an end to their fieldwork, Spaulding said. That hasn't been the case, he said, but field work is different. Researchers often find themselves standing at the site of their fieldwork flying drones, then ground-truthing what the drones detected. A drone may find a raptor nest, but researchers still have to climb up to see what's inside.

"I can see they will replace certain aspects and enhance others," Spaulding said. Biologists using drones "may not be tracking animals anymore," he said, "but there's still a lot of work to do."

▼ **A researcher uses a drone to monitor prairie dogs in Theodore Roosevelt National Park in North Dakota.**

Credit: M. Thompson

Behind the desk

The loss of field time worries Denver Holt, president of the Owl Research Institute in Montana. "I saw that when I started," he said. "If you're lucky enough to get a job, you're likely to spend most of your career behind a desk." Writing in *The Wildlife Professional* last year, Holt argued that it takes 10,000 hours of rigorous study—in the field—to become a good field biologist.

Holt said noninvasive techniques and new technology are limiting the time biologists get to spend in the field. His team is using automated recorders to conduct owl surveys, so they don't have to go out at night. "They're getting good results, no doubt about that," he said. "But I chuckle and say I just can't imagine doing owl surveys without going out and doing surveys—hearing the sounds of the night, the smells, and interactions or chance encounters with other wildlife. The experiences, the learning, there are all kinds of things we're missing."

The technology is beneficial, Holt said, but it needs to be complemented by field research, where observation has always been a critical noninvasive technique. "This is perhaps our most important wildlife research tool," he said. "To me, it is very clear—when talking with researchers or reading papers—who has real experience with the animals and habitats they talk about. Personal experiences. That's ecology."

But Ben Weinstein, a research scientist at the University of Florida who has used satellite data to answer questions about wildlife, said these technological advances are simply opening up time for researchers to answer questions rather than collect data. “There’s this narrative in the wider world that it’s always humans versus computers,” he said. “That couldn’t be more false. Instead, think about it as humans collaborating with computers, so computers can perform those tasks that are laborious or dangerous and allow people to use their brains for what’s truly needed.”

Credit: Miriam Zemanova

▲ **Miriam Zemanova’s field assistant Raquel Lázaro swabs a frog’s skin to collect the amphibian’s DNA and to detect pathogens like *Batrachochytrium dendrobatidis*.**

The 3Rs

For biologists, choosing the right technique comes down to what questions they are trying to answer. Sometimes, handling an animal is the only way to gather the data they need. But other times, handling can affect the data. Collaring and tagging can be stressful for wildlife, which can affect the results. And while no one likes to see an animal injured or accidentally killed by a trap or a mist net, the stakes are greater when handling threatened or endangered species, where losing an individual can have an outsize effect for the population.

Researchers using helicopter net-gunning on white-tailed deer (*Odocoileus virginianus*) and pronghorn (*Antilocapra americana)* wondered if the technique was causing mortalities (Jacques et al. 2010). They found that of net-gunned pronghorn, almost 9% died from capture-related injuries and 1.4% of deer died from injuries during helicopter captures. The researchers recommended limiting pursuit time and making other changes to decrease these effects.

Miriam Zemanova, a researcher at the Environmental Sciences and Humanities Institute at the University of Fribourg in Switzerland, encourages researchers to pursue the least invasive techniques possible to get the answers they’re looking for.

She became alarmed as an undergraduate after learning that researchers often marked frogs by cutting off toes. During her own PhD research, she had a nagging feeling that she could cause her research subjects less harm. When she learned about the 3R’s—replacement, reduction and refinement—an approach first proposed about 60 years ago for animal testing in laboratory research, she wondered if they could be applied to wildlife research.

In the lab, the 3Rs can mean testing a new drug in a cell culture instead of with a mouse or improving the housing conditions of lab animals. “This would, of course, not work in wildlife research,” she said. But she felt that something like it might work to guide wildlife research.

She set out to adapt the 3Rs to wildlife research—maybe replacing captured wildlife with computer models when appropriate, or reducing the sample size, or refining the process by taking skin swabs instead of blood samples (Zemanova 2020). She created the website 3rswildlife.info to share possibilities.

When she surveyed ecologists, she found only 39% knew the 3Rs principles, and only 23% used noninvasive research methods (Zemanova 2021). But that’s beginning to change, she said, as new methods and technologies emerge.

“I think we would be amazed at the creativity in techniques wildlife biologists can come up with,” she said. ■

TWS MEMBER **Dana Kobilinsky** is the associate editor for The Wildlife Society.