eDNA & the Search for Hellbenders

For the last 6 years, Zoo staff and Lori Williams, Mountain Wildlife Diversity Biologist for the NC Wildlife Resources Commission, have searched for Hellbenders among the state's mountain waterways. The search is important, both to better define this elusive amphibian's range and to assess the health of its remaining populations.

Staff and volunteers conduct these surveys by wading into fast-running, clear and cold mountain streams and turning over rocks, large and small, to look for Hellbenders. The work can be grueling, as researchers, dressed in waders or wet suits, brace against the frigid, swift currents.

It takes a team to flush out Hellbenders. Someone has to work a logging tool, called a "cant hook" or "log peavey," under the larger rocks and pry them up enough for a snorkeler to dive down to look and feel for Hellbenders. Other snorkelers work alone, sifting through small rocks and cobbles in search of Hellbender larvae and juveniles.

Together, our teams inch through streams, capturing every Hellbender we can to weigh it, estimate its age, assess its health and gently set it back under the rock or cobble where we found it. In the fall, we expand the searches to look for Hellbender eggs. Male Hellbenders make this task a bit easier by guarding the rocky nests where their mates have deposited their fertilized eggs in a shallow pit.

And so we go, from May through October, traveling from one tributary to the next to survey the streams and the creeks that define the five North Carolina mountain river systems where Hellbenders live. As weather and water conditions shift, our successes wax and wane. But, we keep going, visiting places where Hellbenders were recorded in the past and places where no records of Hellbenders can be found. We document our findings, noting the size, the health and the reproductive status of every population we discover.

North Carolina's Hellbenders

We undertook this expedition because most experts believe that North Carolina shelters one of the world's healthiest remaining Hellbender populations. But, without any surveys, no evidence exists to support these claims.

Even a quick glance at a state map hints to the enormity of this task. A tangled network of rivers, streams and creeks crisscrosses the North Carolina mountains. Our work suggests that some 2,000 to 2,500 of these waterways might support Hellbenders. Searching every potential habitat is a Herculean task that will require at least 5 more years of work. When we finish, we should understand the range, the health and the condition of the state's Hellbender population and have a good grasp of which segments of this population are stable and which are increasing or in decline.

Because we test water quality at each survey site, our research will also quantify the health of the mountain's waterways. Hellbenders, along with several other aquatic species, are especially sensitive to water quality, so identifying declining populations can alert officials to issues that may threaten the state's drinking water or harm its recreational waterways. Data from our surveys have already helped several mountain river systems receive High Water Quality status from the NC Division of Water Quality.

STEVE ATKINS

Attuned to the Welfare of Wildlife

Because our survey methods may cause minor disruptions or short-term stress to waterways or to wildlife, especially when we explore nesting places, we follow a strict protocol to minimize our impact. We turn, and return, rocks carefully and handle captured Hellbenders gently as we weigh, measure and resettled them under their chosen rocks. When we release captured Hellbenders, we observe their behaviors. Typically, our study Hellbenders scurry back under their rocks and resume normal behaviors immediately. When we have checked the status of study Hellbenders a day or two after their capture, we have found them living normally, with no signs that they or their habitats had been disrupted.

While our studies seem to have little impact on Hellbenders, the work takes a toll on our researchers. It takes time, energy and money to find and explore the mountain streams that may harbor Hellbenders. Consequently, our team was excited to learn about an experimental technique that may ease the burdens associated with finding Hellbender inhabited streams. If this technique pans out, it will make it easier and quicker to find Hellbenders and will

nearly eliminate the potential for stressing wild Hellbenders or disturbing their habitats.

This new technique will send researchers looking for Hellbender DNA, not Hellbender bodies, to determine the species' presence or absence in a waterway. With no rocks to turn or currents to forge, researchers will exchange their peavies for bottles and will scoop water, not Hellbenders, out of the torrents. Once captured, the water samples will be pumped through filters designed to trap broken bits of DNA—the molecule that carries an organism's genes—swirling around in the liquid. Known as "aquatic environmental DNA," or "eDNA" for short, these snippets of DNA end up in waterways as the jetsam and flotsam of sloughed-off skin cells and excreted feces and urine.

Hellbender Forensics

The trick to using eDNA depends on devising a test that can pick the target species' (in our case Hellbender's) DNA, out from all the other DNA residue that fish, spiders, deer, maple trees, researchers, mosquitoes and such, leave behind when they pass through the waterway.

This task gets dicey because all life on Earth shares an evolutionary history and, consequently, some common ancestors. A few genes from these ancient, common ancestors continue to occupy space on the chromosomes of every living organism. This shared inheritance means that much of the DNA snagged by a water filter cannot distinguish a rat from a person or a pineapple or a mushroom. Regardless of their species or their Kingdom of origin, Earth's life forms share stretches of identical DNA.

So, to use eDNA to test for the presence of Hellbenders, researchers must first find a short section of DNA that is unique to Hellbenders. Once that snippet is known, researchers can build a complementary molecule, called a "primer" that can be produced in large quantities and sent out in large batches to find and latch onto any, and only, matching bits of Hellbender DNA.

If a primer finds its complement, researchers can use an enzyme to copy that snippet over and over and over. Eventually, enough copies pill up to be easily detected in the sample. The process that drives the primer to bond with and the enzyme to magnify the target DNA is called a "polymerase chain reaction." It is complicated, but it eventually produces millions of copies of the DNA target.

(To learn more about genes and this process, visit Cold Spring Harbor Laboratory's Website at http://www.dnalc.org/websites/dnaftb.html and click on http://www.dnaftb.org/on the right side of that page.)

European researchers were the first to apply this technique to amphibians, using the polymerase chain reaction to find bits of DNA left behind by members of an invasive species: the Bullfrog. Later, other studies found traces of DNA shed by an invasive Asian carp and several amphibian species.

A review of this research inspired me to look for a research partner who would be willing to help us apply this inexpensive, efficient technique to our Hellbender studies in the mountains. My search led me to Dr. Stephen Spear of the Orianne Society in Georgia and an associate of the University of Idaho. He helped us develop a study that uses eDNA to look for Hellbenders, and we are experimenting with using the same technique to search for Mudpuppy DNA in the same streams.

STEVE ATKINS

Research Goals

Our study will apply eDNA techniques to test for the presence, or absence, of Hellbenders and Mudpuppies in various streams and to determine if the testing can provide information on the density of identified populations, the effects that stream flow rates have on quantifying eDNA and the effectiveness of using a series of samples to assess the reproductive status of Hellbender populations. Positive findings for any or all of these questions will enhance our capacity to understand and ultimately protect these highly secretive, but environmentally important, animals.

We expect to encounter some challenges as we adopt this technique to the special environmental and species needs of Hellbenders and Mudpuppies. We believe, however, that we can work out these problems in the field.

What We Plan

We will look at several locations with known Hellbender and/or Mudpuppy populations, and we will survey waterways where these species have not been recorded. We will also test several streams outside the range of our study species to confirm that the DNA primers are not producing false positives. Because this technique is relatively inexpensive, if successful, it will allow us to sample more waterways in less time than would be possible with our current methods. This new study, if successful, will also benefit other researchers working with Hellbenders and may inspire them to test this technique with other aquatic species.

This study and our other field surveys rely on funding from NC Zoo Society members, and we hope to be able to continue this important work in monitoring our native natural resources. Stay tuned for the results of this important research!

JOHN D. GROVES, CURATOR OF AMPHIBIANS AND REPTILES