STATE WILDLIFE GRANT—INDIANA
Efficacy of Using Environmental DNA (eDNA) to Detect Kirtland's Snake

A Kirtland's snake found beneath a coverboard in October. (Photo by Rikki Ratsch)

CURRENT STATUS

Second year of a two-year project

FUNDING SOURCES AND PARTNERS

State Wildlife Grant Program (T7R20)
Purdue University Fort Wayne

PROJECT PERSONNEL

Dr. Mark Jordan, Principal Investigator, Purdue University Fort Wayne
Rikki Ratsch, Graduate Student, Purdue University Fort Wayne

BACKGROUND AND OBJECTIVES

The Kirtland's snake (*Clonophis kirtlandii*) is a small, secretive snake that is state-endangered in Indiana. It occurs in moist soil habitats near permanent or temporary bodies of water that tend to support crayfish populations. Kirtland's snakes use burrows created by crayfish and other animals. The species is most often found at the surface during moist conditions in spring and fall. The semi-fossorial (i.e., sometimes living underground) and secretive nature of the Kirtland's snake make it difficult to find. There have been few sightings in the state during the past decade even though Indiana is at the center of the species' geographic range. The difficulty to find and properly survey for this snake presents a major challenge to understanding its status. Environmental DNA monitoring has the potential to fill this gap and provide an effective way to assess the distribution of this species.

Environmental DNA, or eDNA, is non-living biological material that is shed from animals and accumulates in the environment. It originates from biological functions like waste excretion, shedding of tissue, or other bodily secretions. Genetic material present in such biological material can be removed from collected samples of water or soil. The detection

An adult Kirtland's snake captured in July. The Kirtland's snake is a small species, rarely exceeding two feet in length. (Photo by Rikki Ratsch)

and quantification of this genetic material provides indirect evidence for the presence of a particular animal in a sampled habitat. The advantage of eDNA is its ability to detect the presence of an animal without the need to capture and handle it. The endangered status, challenges in detection, and limited knowledge of the Kirtland's snake make it an ideal candidate for this technique. The objectives of this project are to:

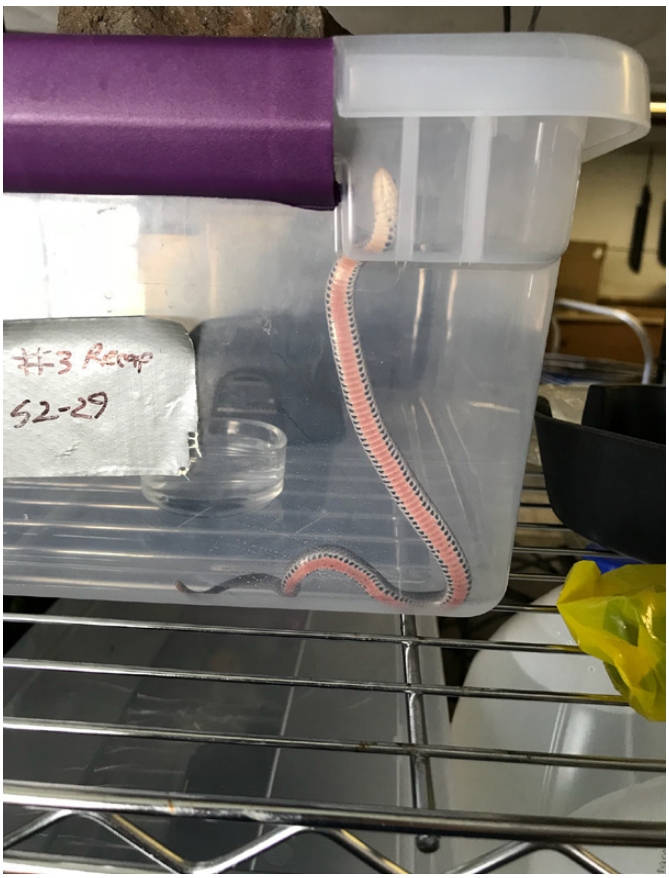
1. Develop an eDNA assay that is specific to the Kirtland's snake and excludes other species.
2. Determine if Kirtland's snake eDNA is readily detectable and quantifiable.
3. Establish the degradation rate of Kirtland's snake eDNA in the environment.
4. Develop an eDNA sampling protocol to use in Kirtland's snake surveys.

METHODS

Quantitative polymerase chain reaction (qPCR) is a highly sensitive method for copying short, specific strands of DNA in a sample. Using DNA isolated from snakes, regions within the mitochondrial genome that target the Kirtland's snake and exclude other species were tested. Once a species-specific region was identified with the greatest copying efficiency, we screened environmental samples. Using qPCR can not only detect the presence of DNA in the sample, but also quantify the amount of DNA. This can provide insight into what sample localities, types, and collection times are most effective and the overall sensitivity of the Kirtland's snake eDNA assay.

A wildlife refuge in southern Indiana known to support Kirtland's snakes was chosen for weekly coverboard surveys to establish relative snake abundance at 11 sites. Ventral scale clipping was used to mark captured snakes and collect tissue samples. Excluding 10 individuals at the start of each field season that were used to collect fecal samples, snakes were immediately released after clipping. Individuals kept for feces collection were housed for two days in plastic containers with air vents, water, shelter, and a heat pad for thermoregulation. After producing a fecal sample, snakes were released at their point of capture.

Habitat data were also collected at every other coverboard to determine if any habitat variables had an influence on Kirtland's snake abundance. Data included canopy cover, ground cover, and distance to landmarks. Canopy and ground cover measurements were collected in the four cardinal directions and their



Kirtland's snakes were temporarily held in captivity to collect feces to test for the persistence of eDNA. The species has a distinct red belly with a row of black dots on either side. (Photo by Rikki Ratsch)

An artificial crayfish burrow made of PVC pipe and buried in the ground. These devices were used for testing the degradation rate of Kirtland's snake DNA in the environment. (Photo by Rikki Ratsch)

A syringe with attached rubber hose used to collect water samples from natural and artificial crayfish burrows. (Photo by Rikki Ratsch)

percentages were averaged. Ground cover categories included water, rock, bare soil, leaf litter, grass, herbaceous vegetation, and shrub. Landmarks were measured up to 30 meters from the coverboard and included distance to the nearest shrub, tree, and log.

Two sites, one of high and one of low snake abundance, were selected for environmental sampling based on the coverboard surveys. Environmental samples, consisting of 10 replicates of four sample types (i.e., crayfish burrow water, crayfish burrow soil, open water, soil beneath coverboards) were collected at each site, producing 80 samples per sampling event. Environmental samples were collected three times during the first field season, once during spring, summer, and fall. A fourth sampling of just crayfish burrow water occurred in spring of the second field season, and 40 samples were collected. Crayfish burrow water was obtained using a large syringe attached to a length of plastic tubing. Open water samples were collected by immersing a plastic bottle in water. Soil from crayfish burrows and beneath coverboards was collected using plastic spatulas. All sampling was conducted using best practices to minimize cross-sample contamination in the field and included negative controls to test for contamination. A protocol for the collection, extraction, and screening of environmental samples was developed using what was learned from field sample collection and DNA extraction.

Again using coverboard surveys, a third site was selected where no snakes were captured for use in a field eDNA degradation study. At this site, 10 artificial crayfish burrows made of PVC pipes were placed into the ground and allowed to fill with water. Collected Kirtland's snake feces was placed into the artificial burrows, and water samples were collected for up to a month afterward, using the same syringe method described above. Samples were collected before and immediately after feces spiking and at days 1, 2, 3, 10, 17, 25, and 31 of the study.

PROGRESS TO DATE

Coverboard surveys proved to be more effective at detecting Kirtland's snakes than eDNA. On average, three to four snakes were captured per 193 coverboards surveyed, while only a single crayfish burrow water sample from spring 2017 resulted in a positive detection out of the four sampling events (380 total samples). Although we are continuing to optimize the

Habitat at a survey area with a high abundance of Kirtland's snakes. (Photo by Rikki Ratsch)

eDNA assay to maximize its sensitivity, snakes have proven difficult to detect via eDNA. They are covered in scales that are shed all at once, which may limit the amount of DNA lost from their skin compared to the amount lost by mucus-covered amphibians or fish. Kirtland's snakes are also a relatively small snake that may produce small amounts of feces. Additionally, repeated sampling was difficult due to fluctuation in groundwater levels, which caused many crayfish burrows and open-water sites to dry out by July. These are all challenging aspects to overcome when applying eDNA to detect snakes.

The field degradation study found that Kirtland's snake eDNA can last for as much as 25 days inside a burrow; however, the ability to detect it drops off rapidly. At one day after spiking the burrows, there was only a 50% chance of a positive detection and by 10 days, detection had decreased to 10%. This rapid degradation underlines the time-sensitivity of Kirtland's snake eDNA. This short lifespan, however, can be beneficial because a positive detection would be from a snake that has been there very recently, and may still be present nearby.

Coverboard surveys were quite successful at capturing Kirtland's snakes. Weekly coverboard surveys conducted from May to October 2017 resulted in the capture of 134 Kirtland's snakes, of which 22 were recaptures. Weekly surveys from April to August 2018 captured only 20 snakes, including one recapture. Differences in abundance were evident across locations, with a high number of snakes at three of 11 sites. Although we are unsure what large scale environmental factors drive variation in abundance, Kirtland's snakes were more likely to be captured at coverboards with close proximity to shrubs and high amounts of grass and herbaceous vegetation nearby.

Seasonal variation in abundance was also apparent. Most snakes were found in May and June, with much lower numbers occurring from July to October. Additionally, many more snakes were captured in 2017 than 2018. This seasonal variation follows observations in other populations where Kirtland's snakes are thought to be on the surface in the late spring to mate before retreating underground in the drier, hotter summer months. This seasonal peak in abundance may also be tied to higher water levels. Generally, spring is the wettest time of year and the 2017 field season was much wetter than 2018. While it is still unknown where the snakes retreat to in the summer, this pattern of seasonal abundance represented in the coverboard surveys aligns well with previous observations of this snake.

Our study has value in discerning the most effective approach for surveying for this secretive species. While we are continuing to refine the eDNA assay to maximize sensitivity to Kirtland's snakes in the environmental samples already collected, we suggest that traditional coverboard surveys are likely to prove more useful. This study highlights the need for additional research into the behavior of Kirtland's snakes, and the quantity and persistence of its eDNA, to refine and improve the technique for this and other species.

COST: $130,466 FOR THE COMPLETE TWO-YEAR PROJECT