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

A young Kirtland's snake under a coverboard in early October. (Photo by Rikki Ratsch)

CURRENT STATUS
First year of a two-year project

FUNDING SOURCES AND PARTNERS
State Wildlife Grant Program (T7R20)
Indiana University-Purdue University Fort Wayne

PROJECT PERSONNEL
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BACKGROUND AND OBJECTIVES
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 snake uses burrows created by crayfish and other animals. The species is most often found at the surface during moist conditions in spring and fall. The semifossorial (sometimes living underground) and secretive nature of 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 and quantification of this genetic material provides indirect evidence for the presence of a particular animal in a sampled

Two adult Kirtland's snakes under a single coverboard in late June. The species has a distinct red belly with a row of black dots on either side. (Photo by Rikki Ratsch)

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 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 eDNA in the environment.
4. Develop an eDNA sampling protocol to use in Kirtland's snake surveys.

METHODS

A wildlife refuge in southern Indiana known to support Kirtland's snake 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 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.

Two sites, one of high and one of low snake abundance, were selected for environmental sampling using coverboard surveys. Environmental samples, consisting of 10 replicates of four sample types (crayfish burrow water, crayfish burrow soil, open water, soil beneath coverboards) were collected at each site, producing 80 total samples. Environmental samples were collected three times, once each in spring, summer, and fall. 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.

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 are being tested that target Kirtland's snake and exclude other species. Once a species-specific region is identified and has high copying efficiency, environmental samples will be screened. qPCR can not only detect the presence of DNA in the sample, but also quantify the amount of DNA. This will 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 protocol for the collection, extraction, and screening of environmental samples will be developed using what is learned from the 2017 sample collection and DNA extraction. The protocol will be tested in 2018 in areas with recently confirmed presence of Kirtland's

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

snakes. Finally, to determine the field persistence of eDNA, crayfish burrows will be spiked with Kirtland's snake feces and repeatedly sampled through the 2018 field season.

PROGRESS TO DATE

The weekly coverboard surveys conducted from May to October 2017 resulted in the capture of 130 Kirtland's snakes, of which 21 were recaptured. Differences in abundance were evident, with a high number of snakes at three of 11 sites and fewer snakes at the remainder. Seasonal variation in abundance was also apparent. Most snakes were found in May and June, with much lower numbers occuring from July to October. 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. More data are needed to determine where snakes retreat to in summer and what environmental factors are associated with them being found in some areas more than in others.

Despite challenges associated with sampling the same areas over multiple seasons, we collected environmental samples at sites with both high and low Kirtland's snake abundance. Changes in groundwater level complicated repeated water sampling because many crayfish burrows and open-water sites became dry by July and August. Preliminary results suggest early spring may be the best time to collect eDNA samples. Water is more available, and Kirtland's snake activity is at its seasonal peak. The environmental samples will allow testing of this idea and provide overall information on Kirtland's snake eDNA presence and quantity. Screening with qPCR will be conducted this winter and is expected to be completed in spring 2018.

A site with suitable habitat for Kirtland's snakes but lacking their presence will be used for eDNA degradation testing in spring 2018. At this site, Kirtland's snake feces collected in 2017 will be added to several crayfish burrows and followed by repeat environmental sampling. Snake surveys will also continue in 2018 at the 11 study sites that will provide additional mark-recapture data to assess population status and reveal any differences in annual abundance. Additional environmental samples will be taken both at the survey sites and at new areas with recent records of Kirtland's snake to test and refine the eDNA protocol.

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