STATE WILDLIFE GRANT—INDIANA
Snake Fungal Disease Testing in Indiana

A queen snake is swabbed to test for snake fungal disease. (Photo by Seth LaGrange)

CURRENT STATUS

Second year of a three-year project

FUNDING SOURCES AND PARTNERS

State Wildlife Grant Program (T7R22)
University of Illinois
Illinois Natural History Survey

PROJECT PERSONNEL

Dr. Matt Allender, Principal Investigator, Veterinary Clinical Medicine and Comparative Biosciences, University of Illinois

Dr. Sarah Baker, Co-Principal Investigator, Illinois Natural History Survey

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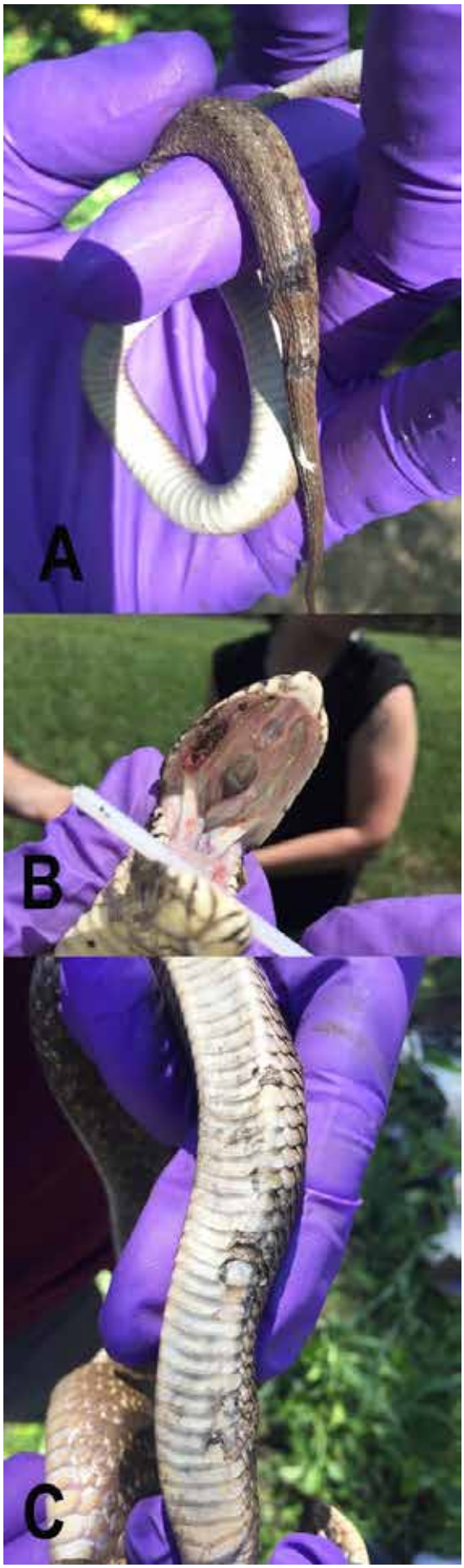
Seth LaGrange, Illinois Natural History Survey

BACKGROUND AND OBJECTIVES

Emerging fungal pathogens are becoming increasingly relevant to the conservation of wildlife populations. Widespread die-offs from pathogens such as white-nose syndrome in bats and chytrid funguses in amphibians have been implicated in population declines and pose a serious threat to many species.

Ophidiomycosis, or snake fungal disease (SFD), is an emerging fungal pathogen in North America. The causative agent of the disease is *Ophidiomyces ophiodiicola*, which can persist in soil and grows at a wide range of temperatures. Ophidiomycosis causes skin lesions ranging from minor scale abnormalities to severe swelling, disfiguration, loss of tissue/bone, and death. The known-fate mortality rate for some species can be greater than 90%. Although the primary mode



of transmission is unknown, it is likely that infection can be passed through contact with the fungus in soil (especially if there are abrasions on the skin), contact with other infected individuals, or from mother to offspring.

To date, ophidiomycosis has been documented in 22 states and in more than 15 genera of captive and free-ranging snakes. Before this study, no targeted sampling had been done in Indiana despite confirmed occurrence of ophidiomycosis in all bordering states. Many of the genera reported to be susceptible to ophidiomycosis occur within Indiana, thereby providing a gap in the understanding of this pathogen and the conservation plans developed. The objectives of this project are to:

1. Determine the occurrence and prevalence of SFD in free-ranging snakes throughout Indiana.
2. Make relevant management and conservation recommendations based on the results.

METHODS

In 2018, all snake captures were made using visual encounter surveys and road cruising surveys, or from dead snakes that people found on roads and submitted to the DNR. All live-captured snakes were given a wellness exam consisting of a behavioral assessment and thorough visual assessment of the skin for lesions or abnormalities. If present, lesion number and location were recorded. Sex, weight, and snout-vent length were determined for all live snakes, when possible. Captured individuals were marked with a scale clip to prevent repeat sampling of the same individual. Skin swabs were collected from all captured snakes regardless of whether lesions were present. Collected swabs were labeled and stored at -20°C until processing.

Collected swabs were tested for presence of *Ophidiomyces ophiodiicola* DNA using quantitative polymerase chain reaction (qPCR). DNA was extracted using a Qiagen DNEasy kit. DNA concentration and purity were determined using a spectrophotometer. The qPCR assays were conducted using a real-time PCR thermocycler, and data were analyzed using the associated software.

PROGRESS TO DATE

One hundred and ninety-six individual snakes of 22 different species have been swabbed from all 10 project regions in Indiana. Northern watersnakes (35 individuals), brown snakes (23), Kirtland's snakes (19), and timber rattlesnakes (16) were the most commonly sampled species.

Quantitative PCR identified 20 positive individuals (13 in 2017, seven in 2018) and 176 total negative

(Left) Clinical lesions suggestive of ophidiomycosis seen in a brown snake (A), bull snake (B) and eastern hognose (C). (Photo by Ellen Haynes)

COMMON NAME	SPECIES NAME	*n*	NUMBER POSITIVE	PERCENT POSITIVE
Northern Copperhead	*Agkistrodon contortrix*	2	0	0%
Kirtland's Snake	*Clonophis kirtlandii*	19	3	16%
Black Racer	*Coluber constrictor*	7	2	29%
Timber Rattlesnake	*Crotalus horridus*	16	0	0%
Ring-necked Snake	*Diadophis punctatus*	8	0	0%
Eastern Hognose Snake	*Heterodon platirhinos*	2	0	0%
Eastern Black Kingsnake	*Lampropeltis nigra*	2	0	0%
Eastern Milksnake	*Lampropeltis triangulum*	7	2	29%
Copperbelly Watersnake	*Nerodia erythrogaster neglecta*	1	0	0%
Northern Watersnake	*Nerodia sipedon*	35	9	26%
Rough Green Snake	*Opheodrys aestivus*	2	0	0%
Gray Ratsnake	*Pantherophis spiloides*	3	0	0%
Eastern Foxsnake	*Pantherophis vulpinus*	4	0	0%
Bull Snake	*Pituophis catenifer*	6	1	17%
Queensnake	*Regina septemvittata*	9	2	22%
Brown Snake	*Storeria dekayi*	23	0	0%
Red-bellied Snake	*Storeria occiptomaculata*	1	1	100%
Western Ribbonsnake	*Thamnophis proximus*	1	0	0%
Plains Gartersnake	*Thamnophis radix*	5	0	0%
Eastern Ribbonsnake	*Thamnophis sauritus*	3	0	0%
Common Gartersnake	*Thamnophis sirtalis*	36	0	0%
Smooth Earthsnake	*Virginia valeriae*	1	0	0%
	Unknown	3	0	0%
TOTAL		**196**	**20**	**10.2%**

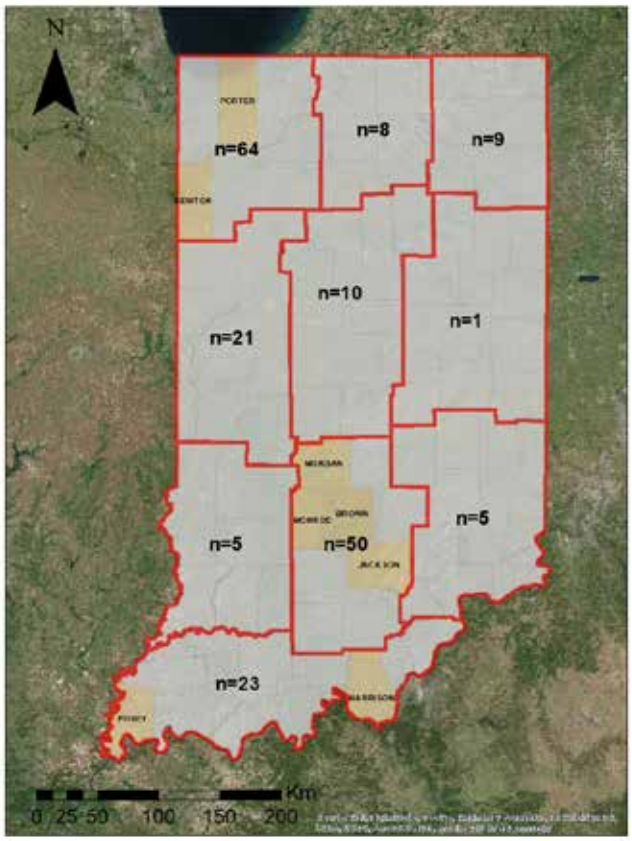
A total of 196 snakes of 22 species were sampled for Ophidiomyces ophiodiicola DNA in Indiana during 2017 and 2018. Positive cases were detected from 20 individuals of the seven species shown in red text.

individuals across the two years. The overall prevalence of individuals positive for *Ophidiomyces* was 10.2%, with a significantly higher prevalence of 24.5% in 2017 compared to 4.9% in 2018. Nine positives (47%) came from the southern region, and eight (42%) originated from the southcentral region. Eight of the positive snakes (42%) were captured in Harrison County, followed by Brown (3; 16%) and Jackson (3; 16%) counties. One positive snake was detected in each of the following six counties: Kosciusko, Monroe, Morgan, Newton, Porter, and Posey.

Nine of the positive cases (45%) were observed in northern watersnakes. Other species less commonly observed with *Ophidiomyces* included the Kirtland's snake (3 positive individuals), black racer (2), eastern milksnake (2), queensnake (2), bull snake (1), and red-bellied snake (1). Nine of the positive cases were male, four were female and seven were of unknown sex. Eight positive cases were adults, seven were juveniles or subadult, three were newborns and two were of unknown age class. Median fungal copy per ng total DNA was 23.6 copies (range: 0.27–3,264.1).

Field work will resume in 2019 to collect the remaining samples to reach the targeted sample size in each region.

COST: $227,457 FOR THE COMPLETE THREE-YEAR PROJECT



Number of snakes sampled for ophidiomycosis by Indiana region, 2017–2018. Positive samples were detected in the eight counties that are shaded orange.