Development and use of a diagnostic assay for detection of *Ophidiomyces* (formerly *Chrysosporium*) in Timber rattlesnakes and Eastern massasaugas

Report prepared by:

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Prepared for:

Illinois Department of Natural Resources

Wildlife Preservation Fund Grant #14-L18W

August 26, 2013 – October 1, 2014

***Project objective as described in the application and grant agreement*:**

The following objectives are critical for describing the disease progression and resolution: 1) develop a sensitive diagnostic test to accurately diagnose this disease in clinical and subclinical snakes; 2) determine the prevalence of disease in timber rattlesnakes and eastern massasaugas in Illinois. Current testing strategies for the eastern massasauga are using less sensitive methods that have limited ability to detect subclinical infections.

***Completed project description*:**

The project achieved its intended goals by developing a highly sensitive and specific test for detection of *Ophidiomyces*, the causative agent of Snake Fungal Disease. Furthermore, it identified new cases that were missed by the previous method. Furthermore, we were able to exceed expectations by testing museum massasaugas. This allowed us to determine the actual emergence of this pathogen as 2000, not 2008 as previously suspected. Samples of Illinois timber rattlesnakes were not tested because they were unavailable in 2013, but the assay was successful in detecting it in this species from other states. The project resulted in data included in 2 manuscripts, one to Journal of Veterinary Diagnostic Investigation focusing on assay development and the other in the Biology of the Rattlesnake, which includes the retrospective analysis.

INTRODUCTION

Studies of wildlife diseases in natural populations aid conservation and recovery of endangered animals. In 2008, three Eastern Massasaugas (*Sistrurus catenatus*) from the Carlyle Lake (Carlyle, IL) population died from a keritinophilic fungal infection with *Ophidiomyces ophiodiicola* (snake fungal disease (SFD); formerly *Chrysosporium ophiodiicola*).[1] Fungal infections in reptiles are typically caused by opportunistic pathogens, infecting animals with a depressed immune system. However, experimental studies show *O. ophiodiicola* can serve as a primary pathogen and source of mortality in reptiles.[1,5] Since 2008, nine Eastern Massasaugas from the Carlyle Lake population have been diagnosed with an *O. ophiodiicola* infection using a conventional polymerase chain reaction (PCR) assay.[1,2] In 2011, health assessments of the Carlyle Lake population focused on early detection of the fungus using conventional PCR on facial swabs, but none of the 38 animals tested were diagnosed as having *O. ophidiicola*.[2] Given that clinical cases of *O. ophiodiicola* are still reported at the site, failure to detect it in 2011 suggests that the current methods may be inadequate and emphasize the need to improve detection methods.

The purpose of this study was to develop and evaluate a real-time PCR (qPCR) assay for characterizing an emerging pathogen, *O. ophidiicola* in snakes. The hypothesis tested in this study was that a real-time PCR assay would be both sensitive and specific for characterizing the epidemiology of *O. ophidiicola* in snakes. This is essential when considering the application of these assays to additional free-ranging populations and/or experimental models that require correct identification of infected individuals prior to management intervention. Furthermore, it allows for investigating and monitoring potential climatic and environmental impacts on the disease, treatment, and management options.

MATERIALS AND METHODS

Dry cotton-tipped applicators (swab) were used to sample the skin or underlying subcutaneous tissue of 52 (38 previously described[2] and 14 additional cases) free-ranging snakes that were presented to the university of illinois at urbana-champaign wildlife epidemiology lab. Nucleic acids were extracted from fresh frozen tissues, formalin fixed paraffin embedded tissues, swabs, or cultures, following 1 hr incubation in 300 u of lyticase, according to the manufacturer's instructions.[a] the quantity and purity of the dna were evaluated by spectrophotometry.[b] conventional pcr was performed in 25 µl reaction volumes using a commercial kit[c] with 200 pm of each primer chrysoits-f and chrysoits-r (table 1), 0.8 mm of total dntps, 0.6 u of *taq* and 2 µl of dna template. The pcr conditions were as follows: denaturation at 94°c for 5 minutes, followed by 30 cycles at 94°c for 30 sec., annealing at 59°c for 30 sec. Then 72°c for 30 sec. With a final extension at 72°c for 4 min. The dna quality and absence of inhibitors were assessed for each sample by demonstrating successful amplification of snake mitochondrial dna using the 16sat and 16sbt primers (4 µl each from10 µm stock) (see table 1), 1 commercial lyophilized pcr reagent bead, and 40 µl sterile distilled h_2o. Following a 3 minute denaturation, 35 cycles of 94°c for 1 min., annealing at 55°c for 1 min, and 72°c for 1 min. Were performed, with a final extension at 72°c for 10 min. The dna quantity per reaction varied from 10 – 100 ng per reaction.

A 414-bp region of the internal transcribe spacer 1 (its1) region that spans between 18s and 5.8s rrna was amplified with the cloning primers (table 1) the pcr products were sequenced in both directions at the keck biotechnology center at the university of illinois, and compared to sequences in genbank using megablast. The pcr products were then cloned in *E. coli* using the cloning primers (table 1).[e] the plasmids were linearized with the restriction enzyme *bam*h1, purified,[f] and quantified using spectrophotometry. Ten-fold serial dilutions of linearized plasmid were made from 2.0 x 10^2 ng/µl to 2.0 x 10^{-7} ng/µl. Fungal genome (dna) copy number was calculated using the following formula:

$$\#\ \text{copies}/\mu l = \frac{(\text{ng dna of plasmid + clone}/\ \mu l)\ (6.022 \times 10^{23}\ \text{copies/mol})}{(\text{bp length})(1 \times 10^{9}\ \text{ng/g})\ (650\ \text{g/mol of bp})}$$

The final copy number for ten-fold serial dilutions ranged from 1.05×10^8 to 1.05×10^1 plasmid copies per reaction. This served as a positive control and for the quantitation of real-time pcr assay.

A minor groove binder dual labeled probe[g] based qpcr assay was designed using a commercial software program.[h] the qpcr assay was performed using the primers ophioits-f and ophioits-r and probe probe-fam (table 1), which targets a 68-bp segment of its1, between the 18s and 5.8s rrna genes of *ophidiomyces*. The qpcr assays were performed using a real-time pcr thermocycler and data was analyzed using the associated software.[i] each 25 µl reaction contained 12.5 µl of 2x commercial supermix,[j] 1.25 µl of the primer-probe (20x), 2.5 µl *O. ophidiicola* target dna, and water to a final volume of 25 µl. The cycling parameters were as follows: 1 cycle at 50°c for 2 minutes followed by 95°c for 10 minutes, then 40 cycles at 95°c for 15 seconds and 60°c for 60 seconds, and a final cycle of 72°c for 10 minutes.

To determine the sensitivity, assays were performed in three technical repeats on dilutions of positive control plasmid of *O.ophidiicola* dna ($1.05 \times 10^8 - 1.05 \times 10^1$ copies per reaction) within a single run in duplicate (6 total reactions). Standard curves were generated using the cycle threshold (c_t) values of the positive control plasmid dilutions. Intra-assay variation was determined for both assays by calculating the mean c_t values, standard deviations, and coefficient of variations separately for each control plasmid dna dilution (table 2). Specificity of the assay was assessed by testing common soil and other fungal organisms (*Aspergillus terreus*, *A. flavous*, *Mucor racemosus*, *Alternaria* sp., *Blastomyces dermatidis*, *Candida albicanus*, *Pseudogymnoascus destrucans*), and found not to result in amplification.

To test the utility in animal samples, 52 free-ranging eastern massasaugas were sampled as part of routine demographic and health assessments.[2] Swabs of the nasolabial pits (on all snakes) and lesions (where present) were collected. The University of Illinois Institutional Animal Use and Care Committee approved all animal use. DNA extracts of swab samples from animals with unknown disease status were evaluated using both conventional (ChrysoITS-F and ChrsyoITS-R primers) and qPCR (OphioITS_f and OphioITS-R primers) and results for detection were compared.

Snakes were captured by conducting visual encounter surveys (VES) during the spring egress in 2013-2014. Snakes were sampled through encounters by U.S. Army Corps of Engineers (COE) and Illinois Department of Natural Resources (IDNR) staff or the public. The site of capture was recorded using a handheld GPC unit, documented behavior, body position, and air temperature at time of capture. Most snakes were processed within a day of encounter, but in

some instances, individuals were held longer. All snakes that were released were places at their site of capture.

Additionally, we were able to examine 261 deceased snakes in the Illinois Natural History Museum collection at the University of Illinois with this new assay. Each snake was examined for evidence of skin lesions, such as swelling, crusts, or nodules. Physical parameters were recorded as above as well as lesion dimensions and photographed. Wedge biopsies were harvested from each lesion separately with a sterilized scalpel blade for each individual lesion, labeled, and placed in an eppendorf tube.

Copy numbers were tabulated and evaluated for normality using the Shapiro-Wilk test. Mean, median, standard deviation, 95% confidence interval, and 10-90% percentiles were determined for positive cases (copy number) for each assay. The Mann-Whitney U test was used to evaluate between assay differences (copy number or DNA concentration). The prevalence of *O. ophidiicola* was determined (categorical variable assigned; 1=positive, 0=negative (based on Ct of lowest dilution of standard curve detected) and binomial confidence intervals were determined for all proportions. Level of agreement (kappa) was determined between both the real-time PCR assays and the conventional PCR based on prevalence. All statistical analysis was performed using statistical software.[k] The seroprevalence for *Ophidiomyces* was determined for Eastern Massasaugas *S. catenatus* from Carlyle Lake, IL. In cases where the prevalence estimate is 0, the 95% confidence intervals were calculated. Fisher's Exact test and Chi square test were used to test associations between all categorical variables (*Ophidiomyces* prevalence, sex, year, age class, behavior, position). WBC, PCV, and TS were compared between SFD positive and SFD negative snakes in 2013 and 2014 using a Mann-Whitney U-test. All data analyses were conducted using in IBM® SPSS® ver. 22 and all significance levels were set at $\alpha = 0.05$.

RESULTS

Serial ten-fold dilutions of positive control plasmids were assessed using the qPCR assay and standard curves were generated based on C_t values. The linear range for the qPCR assay was between 1.05×10^8 to 1.05×10^1 fungal copies with an R^2 of 0.999 (slope = -3.366). The intra- and inter-assay reproducibility was evaluated for the serial dilutions of the control plasmids (Table 2). These results indicate high reproducibility between assays at all dilutions. The dynamic range for the qPCR assay was from 1.05×10^8 to 1.05×10^0. The qPCR assay proved reliable for absolute quantitation of *O. ophidiicola* DNA with greater than 1.05×10^1 fungal copies and relative quantitation for the entire dynamic range.

From 2011, samples derived from free-ranging massasaugas screened for *O. ophidiicola* identified three (6%; 95% CI: 2-16%) positive samples using conventional PCR,[2] while the qPCR identified seven positive samples (13.5%; 95% CI: 55.6-25.8%). The level of agreement between the two assays was moderate (kappa=0.561; 95% CI: 0.191 – 0.931). Each sample was positive for the presence of reptile mitochondrial DNA (data not shown), confirming that the negative *O. ophidiicola* results were not due to the lack of reptile DNA in the sample. For prospective sampling in 2013 and 2014, prevalence was not significantly different between years (2013: 14.3%; 2014: 22.0%). Males (n=12; 21.4%) are significantly more infected than females (n=4; 7.0%) during these 2 years, however most of that variability occurred in 2014 when there

were seven males (30.4%) infected while only one (6.3%) female was infected. There was no location that was significantly associated with infection (p=0.074), however EHSP (33.3%) had the highest prevalence, followed by SSSP (11.1%), Field 3 (10.0%), and Dam East (6.7%). When comparing just SSSP to EHSP, EHSP had significantly more infections than SSSP (p=0.031). Adults (n=16; 18.2%) were significantly associated with infection compared to juveniles (n=0; 0%) (p=0.01).

Retrospectively, *Ophidiomyces* was identified in 11 of the 22 snakes with lesions and as early as the year 2000, with an annual prevalence from 0-29%, which was not significantly different between years (p=0.382) (Figure 1). All positive snakes were from Clinton county. Nine (81.8%) of the positive animals were adult, while two (18.2%) were juveniles, but no significant difference was seen in prevalence between age classes (p=0.238). There was no difference in prevalence between females (n=7; 63.6%) and males (n=4; 36.4%) (p=0.135). There was no difference in monthly distribution of either total cases (p=0.188) or *Ophidiomyces* prevalence (p=0.173) (Figure 2).

The median quantity of DNA recovered from swab samples measured through spectrophotometry was 3.96 ng/µl (10-90 percentiles: 0.12 – 16.17) and median purity A260/280 was 1.75 (10-90 percentiles: 1.46 – 2.00). The median concentration of DNA in samples that were positive for *O. ophidiicola* (20.38 ng/µl) was higher than DNA in swab samples from negative individuals (3.71 ng/ul) (p=0.012). The mean fungal DNA quantity in positive snakes was 26,068 copies (95% CI: 2957 – 49181). The mean DNA copy number in samples positive by both methods was 33,833 (95% CI: 0 – 956671) and was not significantly different from the samples positive only in the qPCR was 20,245 copies (95% CI: 0 – 63250) (p=0.524).

Previous studies have shown that internal controls, ie. a housekeeping gene, may be necessary when evaluating the rRNA gene segment of fungi due to multiple copies within each genome[4] or for quantitation. Those internal controls were not tested in this assay as the objective of the study was to develop a more sensitive assay for detection, rather than absolute quantification. Future studies to determine absolute quantification will need to include these control measures.

The assay had highly reproducible results with intra- and inter-assay variability coefficient of variation of less than 5%. The primers were designed to be specific for the ITS1 segment between the 18S and 5.8S rRNA gene that previous studies have targeted with conventional PCR.[2] While this assay was developed for use in outbreaks involving snakes, the assay has been tested against similar fungal organisms and found to be specific.

DISCUSSION

Ophidiomyces ophidiicola alone or in concert with environmental pressures are threatening free-ranging snakes in the United States, several species of which are under threat or endangered.[5,7,8] Despite the Eastern Massasauga's state endangered status and severity of recent disease outbreaks,[1] the epidemiology of this fungus is not well understood. Current and future epidemiologic surveys that determine the extent of disease and species range depend on effective

diagnostic assays. The current study demonstrated that a qPCR assay is reliable, specific, and sensitive for the detection of the ITS1 region between 18S and 5.8S rRNA genes of *Ophidiomyces*. The qPCR assay has low variability and is not likely to produce a non-specific product and therefore is the recommended assay for snake *O. ophidiicola* surveys. This assay can be used as a tool in the conservation of snakes by identifying emerging and ongoing outbreaks. This assay is more sensitive in detecting fewer fungal copies than conventional PCR, thereby allowing for early, subclinical, or reservoir status detection if those states exist in snakes.

The emergence of *Ophidiomyces* mortalities in the Eastern Massasauga has serious consequences on the sustainability of this species in Illinois. We identified through retrospective surveys of historical specimens the presence of this pathogen as early as 2000 in the Carlyle Lake population. And through the prospective sampling, prevalence of the pathogen causing skin lesions in snakes persists in the population through 2014. However, it is difficult to compare the true prevalences across time due to differences in sampling method (tissue vs swab). The historical specimens may be biased toward individuals thermoregulating on pavement and thus died due to anthropogenic causes (hit by car, etc.). Despite these sampling technique differences, we found no difference in annual *Ophidiomyces* prevalence from 2000 through 2014. There are several years that have fewer than 10 individuals sampled, and no Ophidiomyces detected which results in highly variable confidence intervals and decreases the chances to find statistical significance. However, in the last two years over 50 animals have been sampled and thus the prevalence estimates have less variability and better approach the true prevalence in the population. The persistence in the population is concerning because no Eastern Massasauga that has been identified since 2010 has survived infection more than a year. Thus, in the worst case scenario, prevalence estimates that we observed in prospective sampling indicate a potentially significant single cause of mortality. To mitigate the mortalities of ongoing infections, an understanding of the natural disease ecology of this fungus is needed. This should be multifaceted, but can be initiated with environmental sampling (through eDNA), radiotelemetry of infected snakes, and habitat quality and composition assessments that exist in known Ophidiomyces areas.

As *O. ophidiicola* continues to emerge as a major threat to snakes, it is critical that early and accurate identification of epidemics occur. The approaches to wildlife diseases have historically been to describe outbreaks rather than managing or preventing them, but that is exactly what is needed. This approach will require an innovative and multi-disciplinary team to develop management plans to maintain the health of the population, mitigate current disease threats, and prevent the next major disease threat.

SUMMARY

Fungal pathogens threatening the conservation of wildlife are becoming increasingly common. Disease events threaten the state endangered Eastern Massasauga (*Sistrurus catenatus*) in Illinois. Since the late 2000's, free-ranging snakes across North America have been experiencing a marked increase in prevalence of Snake Fungal Disease associated with *Ophidiomyces ophiodiicola*. Diagnosis has historically relied on histopathology, microbiology, and conventional polymerase chain reaction (PCR). More sensitive methods were needed to adequately characterize the epidemiology. The current study describes the development of a real-time PCR (qPCR) assay for detecting a segment of the Internal Transcribed Spacer 1 region between the 18S and 5.8S rRNA gene. The assay was able to detect as few as 1.05×10^1 copies per reaction. Comparison between conventional and qPCR using 52 swab samples from eastern massasauga rattlesnakes revealed an additional four positive cases using qPCR. The newly developed assay is a sensitive and specific tool for surveillance and monitoring in the conservation of free-ranging snakes. Additionally, we examined 261 deceased snakes in the Illinois Natural History Survey Museum collection (1873-2013). There was an annual prevalence from 0-29% from 2000-2011 in retrospective samples, and prospectively 14.3% in 2013 and 22.0% in 2014. Studies that focus on the wellness of wildlife are helpful in designing and implementing conservation plans and can model future wildlife health studies.

SOURCES AND MANUFACTURERS

a. QIAmpDNA Mini Kit, QiAmp FFPE Tissue Kit, BioSprint 96 automated DNA/RNA extraction, Qiagen, Valencia, CA.

b. EPOCH, BioTek, Winooski, VT.

c. Invitrogen Product# 10342-020, Carlsbad, CA.

d. OmniMix HS bead, Cepheid, Sunnyvale, CA.

e. TOPO TA Cloning® kit, Invitrogen, Carlsbad, CA.

f. QIAfilter plasmid Maxi kit, Qiagen, Valencia, CA.

g. TaqMan® primers, FAM dye labeled, Applied Biosystems, Carlsbad, CA.

h. Primer Express®, Applied Biosystems, Carlsbad, CA.

i. 7500 ABI real-time PCR System, Sequence Detection Software v2.05, Applied Biosystems, Carlsbad, CA.

j. TaqMan Platinum PCR Supermix-UDG with ROX, Invitrogen, Carlsbad, CA.

k. IBM SPSS Statistics 22, Chicago, IL; MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium.

LITERATURE CITED

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Table 1. Primers and Probe for Detection of *Ophidiomyces ophiodiiocola.*

ID	Gene	Sequence	Amplicon
Chryso. ITS-F	ITS1	5'-TGATCCGAGGTCAACCGGAAGAAA- 3'	400 bp
Chryso. ITS-R		5'-TGGAACCGTCAACGAACTCTGTGA- 3'	
16SAT-F[6]	mitochondrial	5'-CGCCTGTTTATCAAAAACA-3'	525 bp
16SBT-R[6]		5'-CCGGTCTGAACTCAGATCACGT-3'	
Cloning - sense	ITS1	5'-GAACGAACTCTGTGAGAAGC- 3'	414 bp
Cloning – antisense		5'-GTACTCCTACCTGATCCGAG- 3'	
OphioITS – F	ITS1	5'-TGTTTCTGTCTCGCTCGAAGAC- 3'	68 bp
OphioITS – R		5' -AGGTCAAACCGGAAAGAATGG- 3'	
Probe – FAM		5' -CGATCGGGCGCCCGTCGTC- 3'	

Table 2. Intra- and inter-assay variability of a real time polymerase chain reaction assay to detect *Ophidiomyces* DNA.*

Interassay				Intrassay			
DNA copy	Ct mean	Ct SD	CV	DNA copy	Ct mean	Ct SD	CV
105000000	15.64	0.04	0.28%	105000000	15.61	0.03	0.16%
10500000	18.26	0.07	0.38%	10500000	18.37	0.03	0.17%
1050000	21.60	0.14	0.65%	1050000	21.75	0.04	0.19%
105000	24.99	0.14	0.55%	105000	25.20	0.05	0.20%
10500	28.24	0.26	0.92%	10500	28.67	0.11	0.40%
1050	31.57	0.23	0.74%	1050	32.09	0.25	0.79%
105	33.04	0.92	2.79%	105	35.70	0.41	1.14%
10	35.86	1.43	3.99%	10	37.67	1.03	2.73%
1				1	38.40	0.17	0.45%
NTC	Undetermined			NTC	Undetermined		

* Ct = Cycle threshold; SD = standard deviation; CV = coefficient of variation; NTC = no template control.

Figure 1: Fungal culture of *Ophidiomyces ophidiicola* grown at University of Illinois College of Veterinary Medicine Diagnostic Laboratory. Used in plasmid development of the qPCR assay.

Figure 2. Standard curve generated from serial dilutions of Ophidiomyces ITS1 gene used to detect Snake Fungal Disease in Eastern Massasaugas.

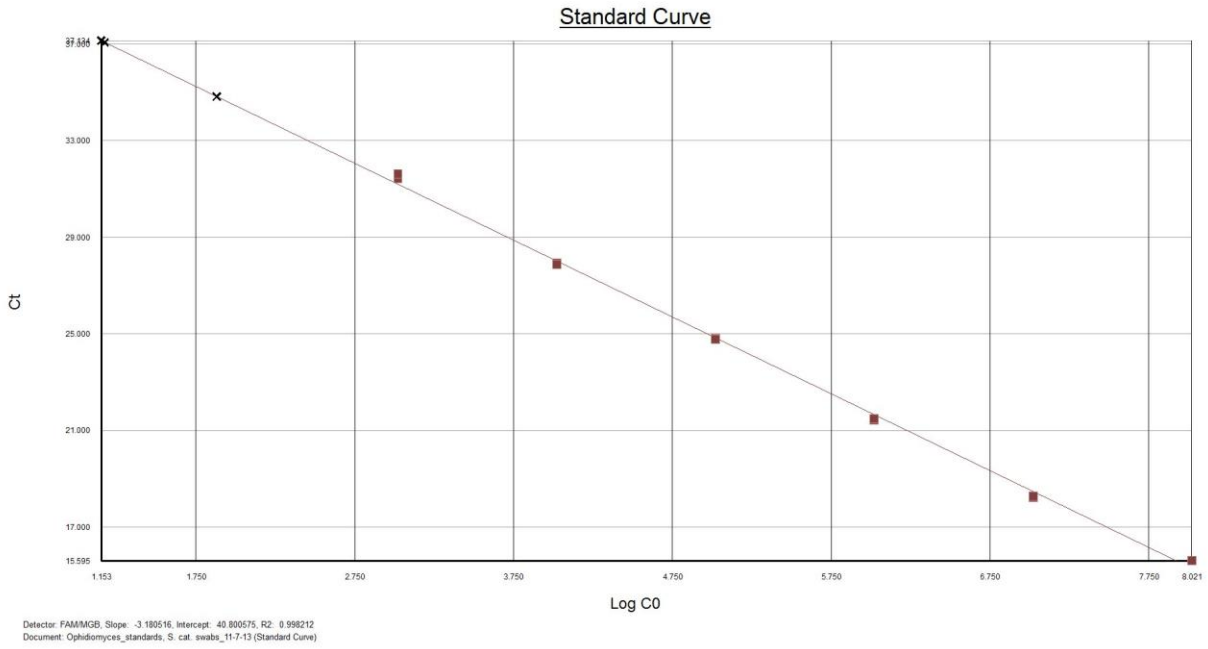


Figure 1: Prevalence of skin lesions and SFD in Eastern Massasaugas (*Sistrurus catenatus*) from Carlyle Lake using collections based snakes from 2000 – 2011 and swabs from 2012 – present.

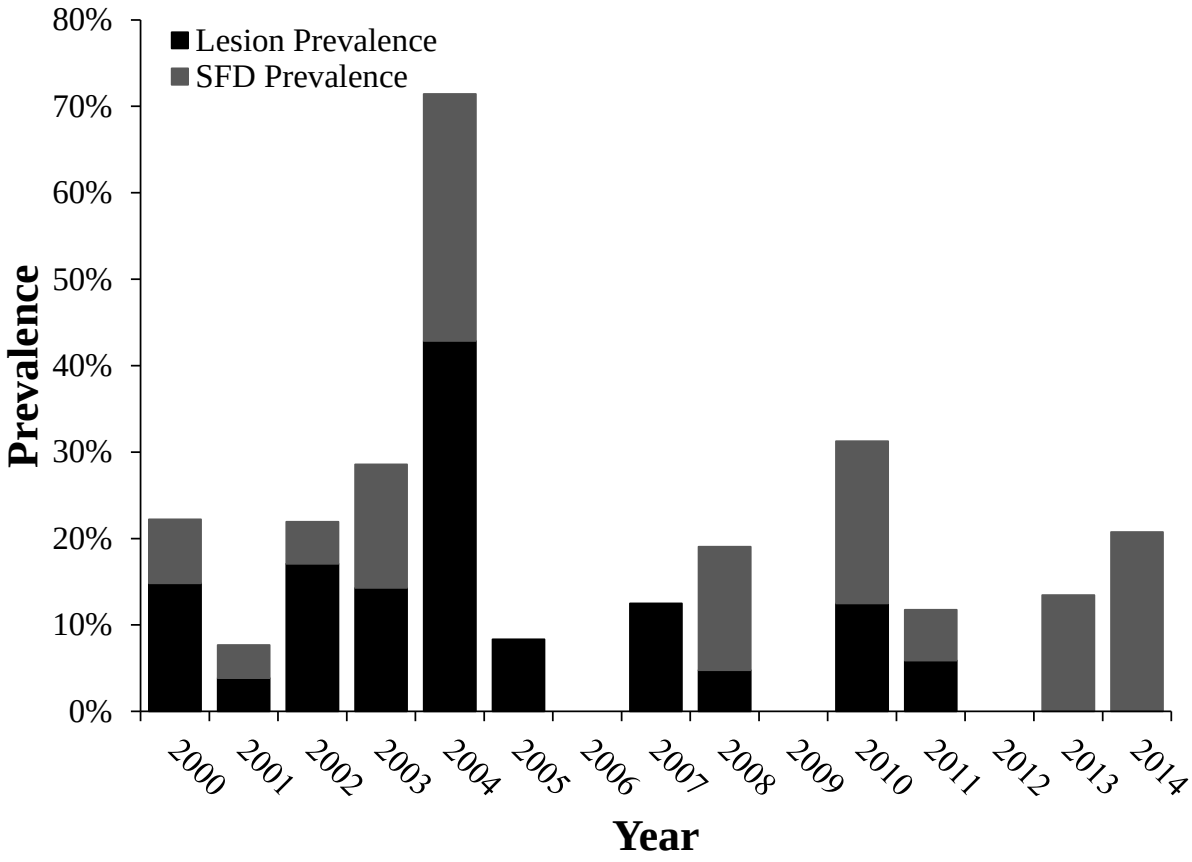
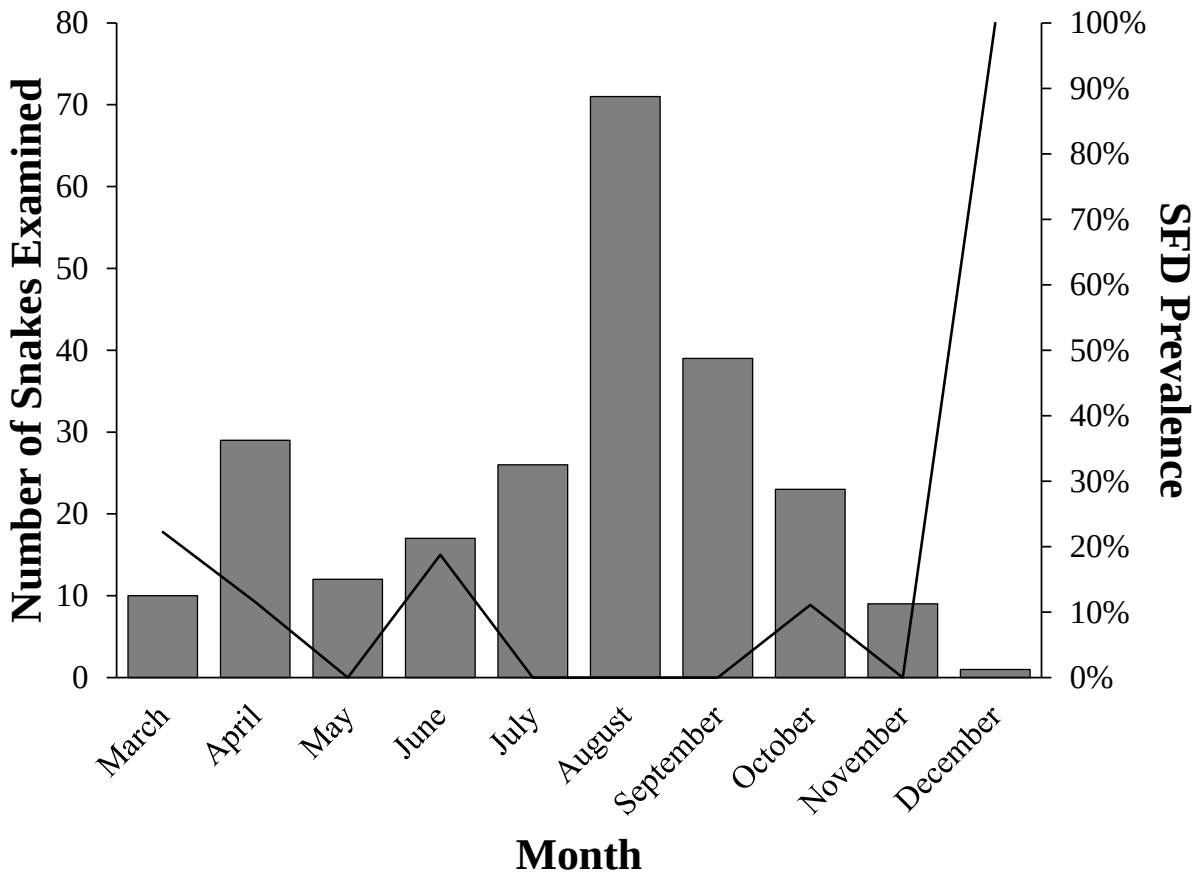


Figure 2: Monthly distribution of deceased Eastern Massasaugas (*Sistrurus catenatus*) from Carlyle Lake in the INHS Museum collection (solid bars; primary axis) and prevalence of *Ophidiomyces* (line; secondary axis) in Illinois from 2000 through 2011.



IDNR
Wildlife Preservation Fund Grant #14-L18W
Development and Use of a Diagnostic Assay for Detection of Chrysosporium in Timber Rattlesnakes and Eastern Massasaugas
Dr. Matthew Allender, Assistant Clinical Professor, University of Illinois at Urbana-Champaign
Expenditure Report

Category	Vendor Name	Vendor Address	Desciption	Qty	Date of Purch	Inv #	Total
Direct Spending:							
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 3 L	1	10/1/2013	IE242483	$ 2.88
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 3 R	1	10/1/2013	IE242483	$ 2.88
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 1 L	1	10/1/2013	IE242483	$ 3.20
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 1 R	1	10/1/2013	IE242483	$ 3.20
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 2 L	1	10/1/2013	IE242483	$ 3.20
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Ophidiomyces Primers # 2 R	1	10/1/2013	IE242483	$ 3.20
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Platinum Quantitative PCR SuperMix-UDG w/ROX	2	10/2/2013	IE236388	$ 1,979.45
Laboratory/Scientific Supplies	Qiagen Inc.	27220 West Turnberry Lane, Suite 300, Valencia, CA 91355	Qiagen Q9.13.13 I5234540 - Protease (30 AU)	1	10/7/2013	I5234540	$ 213.75
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Platinum Quantitative PCR SuperMix-UDG w/ROX	2	11/13/2013	IE254772	$ 1,979.45
Laboratory/Scientific Supplies	Life Technologies Corporation	3175 Staley Road, New York, NY 14072	Assay Id AI5IQI6 Custom TaqMan Gene Expression Assay, LG Scale L: 2900 rxns	1	11/14/2013	IE255254	$ 945.00
Laboratory/Scientific Supplies	Life Technologies / Invitrogen	3175 Staley Road, New York, NY 14072	Eco RI 5000 UN & BAMHI 200 UN	1 & 1	11/20/2013	J1982220	$ 55.41
							$ 5,191.62
Academic / Grad Hourly Wages	**Grace Anne Archer**	**UI Student Employee (FERPA covered)**	**Lab and Field Assistance**				
Academic / Grad Hourly Wages	HR Payroll 2013 BW 24 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		11/20/2013	F0258729	$ 152.90
Academic / Grad Hourly Wages	HR Payroll 2013 BW 25 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		12/4/2013	F0259564	$ 166.10
Academic / Grad Hourly Wages	HR Payroll 2013 BW 26 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		12/18/2013	F0260426	$ 61.60
Academic / Grad Hourly Wages	HR Payroll 2013 BW 27 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		12/31/2013	F0261181	$ 126.50
Academic / Grad Hourly Wages	HR Payroll 2014 BW 2 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		1/29/2014	F0263208	$ 214.50
Academic / Grad Hourly Wages	HR Payroll 2014 BW 3 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		2/12/2014	F0264063	$ 55.00
Academic / Grad Hourly Wages	HR Payroll 2014 BW 4 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		2/26/2014	F0265080	$ 55.00
Academic / Grad Hourly Wages	HR Payroll 2014 BW 5 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		3/12/2014	F0266037	$ 55.00
Academic / Grad Hourly Wages	HR Payroll 2014 BW 7 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		4/9/2014	F0267717	$ 13.20
Academic / Grad Hourly Wages	HR Payroll 2014 BW 8 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		4/23/2014	F0268741	$ 44.00
Academic / Grad Hourly Wages	HR Payroll 2014 BW 9 0	UI Student Employee (FERPA covered)	Lab and Field Assistance		5/7/2014	F0269596	$ 44.00
Workers Compensation	IC151 - WC UIUC Trust 1	UIUC Worker's Comp Trust 1	Worker's Comp at .14% of pay				$ 1.38
							$ 989.18
Academic / Grad Hourly Wages	**Elena Dzhaman**	**UI Academic Hourly Employee**	**Lab Technician**				
Academic / Grad Hourly Wages	HR Payroll 2014 BW 10 2	UI Academic Hourly Employee	Lab Technician		6/12/2014	F0273047	$ 647.50
Academic / Grad Hourly Wages	HR Payroll 2014 BW 11 2	UI Academic Hourly Employee	Lab Technician		6/12/2014	F0273049	$ 814.00
Academic / Grad Hourly Wages	HR Payroll 2014 BW 11 3	UI Academic Hourly Employee	Lab Technician		7/22/2014	F0277029	$ (814.00)
Academic / Grad Hourly Wages	HR Payroll 2014 BW 11 4	UI Academic Hourly Employee	Lab Technician		7/22/2014	F0277029	$ 613.94
Academic / Grad Hourly Wages	HR Payroll 2014 BW 8 2	UI Academic Hourly Employee	Lab Technician		6/12/2014	F0273045	$ 301.30
Academic / Grad Hourly Wages	HR Payroll 2014 BW 9 2	UI Academic Hourly Employee	Lab Technician		6/12/2014	F0273046	$ 740.00
Social Security without Medicare		UI Academic Hourly Employee	SS without Medicare at 6.20% of pay				$ 142.77
Medicare Matching		UI Academic Hourly Employee	Medicare matching at 1.45% of pay				$ 33.39
Workers Compensation	IC151 - WC UIUC Trust 1	UIUC Worker's Comp Trust 1	Worker's Comp at .14% of pay				$ 3.22
							$ 2,482.12
Indirect Cost Recovery			UI State of Illinois Rate of 10% of all expenditures				$ 866.29
Total Direct and Indirect							**$ 9,529.22**
Match Spending (in 873222, 1-200250-873000-873105):							
Academic / Grad Hourly Wages	**Elena Dzhaman**	**UI Academic Hourly Employee**	**Lab Technician**				
Academic / Grad Hourly Wages	HR Payroll 2014 BW 1 0	UI Academic Hourly Employee	Lab Technician		1/15/2014		$ 423.25
Academic / Grad Hourly Wages	HR Payroll 2014 BW 2 0	UI Academic Hourly Employee	Lab Technician		1/29/2014		$ 832.50
Academic / Grad Hourly Wages	HR Payroll 2014 BW 3 0	UI Academic Hourly Employee	Lab Technician		2/12/2014		$ 860.25
Academic / Grad Hourly Wages	HR Payroll 2014 BW 4 0	UI Academic Hourly Employee	Lab Technician		2/26/2014		$ 860.25
Academic / Grad Hourly Wages	HR Payroll 2014 BW 5 0	UI Academic Hourly Employee	Lab Technician		3/12/2014		$ 906.50
Academic / Grad Hourly Wages	HR Payroll 2014 BW 6 0	UI Academic Hourly Employee	Lab Technician		3/26/2014		$ 804.75
Academic / Grad Hourly Wages	HR Payroll 2014 BW 7 0	UI Academic Hourly Employee	Lab Technician		4/9/2014		$ 703.00
Social Security without Medicare		UI Academic Hourly Employee	SS without Medicare at 6.20% of pay				$ 334.21
Medicare Matching		UI Academic Hourly Employee	Medicare matching at 1.45% of pay				$ 78.16
							$ 5,802.87