Evaluating the role of Zoo Campus Environments in Modulating Wild Snake Body Condition and Ophidiomycosis Risk

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Abstract.—Snake Fungal Disease (SFD), caused by Ophidiomyces ophidiicola, poses a growing threat to snake populations. This study compared infection prevalence, body condition, and species composition between snakes found at Caldwell Zoo located in Tyler, Texas and those found in surrounding wild areas. While infection rates were similar (14.3% zoo, 12.1% wild), zoo-caught snakes had significantly higher body condition indices, likely due to more stable resources. Species composition also differed, with zoo population dominated by a few common species, while the wild sample was more diverse. These findings suggest that habitat context influences both health and community structure, with implications for SFD management.

Resumen.— La enfermedad fúngica de las serpientes (SFD), causada por Ophidiomyces ophidiicola, representa una amenaza creciente para las poblaciones de serpientes. Este estudio comparó la prevalencia de infección, el índice de condición corporal y la composición de especies entre serpientes registradas en el Zoológico Caldwell, ubicado en Tyler, Texas, y aquellas encontradas en áreas silvestres circundantes. Si bien las tasas de infección fueron similares (14.3 % en el zoológico, 12.1 % en el medio silvestre), las serpientes capturadas en el zoológico presentaron índices de condición corporal significativamente más altos, probablemente debido a una mayor estabilidad en la disponibilidad de recursos. La composición de especies también difirió: la población del zoológico estuvo dominada por unas pocas especies comunes, mientras que la muestra silvestre fue más diversa. Estos hallazgos sugieren que el contexto del hábitat influye tanto en la salud como en la estructura de la comunidad, con implicaciones para la gestión de la SFD.

Key words: BCI; Caldwell Zoo; metapopulation; snake fungal disease

Ophidiomycosis, commonly known as Snake Fungal Disease (SFD), is an emerging infectious disease impacting snake populations across large portions of the world (Allender, et al.,2015). First described in 2006, SFD is caused by the fungal pathogen *Ophidiomyces ophidiicola* and presents with a variety of clinical and behavioral symptoms (Rajeev, et al.,2009). Clinically, affected snakes may exhibit granulomatous lesions, abnormal shedding (dysecdysis), ulcerations, and other dermal abnormalities; Fig. 1). These symptoms are often accompanied by behavioral changes, such as lethargy, increased basking, and reduced appetite, which can ultimately lead to death and population declines in certain areas (Allender, et al.,2011).

As with other devastating fungal pathogens, such as Batrachochytrium dendrobatidis in amphibians and *Pseudogymnoascus destructans* in bats SFD poses a serious threat to native wildlife(Blehert, et al.,2009, Fisher, et al.,2012). Several species, including Eastern Massasauga Rattlesnakes (*Sistrurus catenatus*) and Timber Rattlesnakes (*Crotalus horridus*), have already been negatively affected by this disease (Allender et al., 2011; Blehert et al., 2009; Fisher et al., 2012).

As wild snake populations face increasing threats, zoological institutions are playing an expanding role in species conservation, offering refuge, breeding opportunities, and public education. However, zoo environments are not impervious to intrusion by wild snakes. These individuals may enter zoo grounds or enclosures, where they can become both a concern and a curiosity. On one hand, they may pose risks through predation or disease introduction; on the other hand, they may also benefit from access to stable food sources, reduced predation risk, and relatively protected environments. This raises the possibility that snakes inhabiting zoo spaces may experience different health outcomes than their counterparts in nearby wild areas. In this study, we compare SFD infection prevalence, body condition, and species assemblages of snakes found on zoo grounds vs those found in surrounding wild habitats, to test whether the zoo campus environment alters disease risk and snake health. We hypothesize that these body conditions and infection rates found in these zoo-caught snakes will be different than those that are found in other wild populations in Texas and around the United States of America (Allender, et al.,2020, Lizarraga, et al.,2023). To investigate these questions, we conducted a comparative field study as follows.

MATERIALS AND METHODS

Snake Sampling. — From May 2023 to May 2024, staff at the Caldwell Zoo in Tyler, Texas routinely collected wild snakes that come on campus as part of their standard procedures (N=112). Our research team was then granted access to sample and measure these individuals. Collected measurements included mass, snout-to-vent length (SVL), head length, and UV reflectivity. Using this data, we calculated BCI using the Quételet index (mass/snout-to-vent length^2). We swabbed each snake's head and body using a sterile, pre-moistened swab with sterilized deionized water. The swabs were stored in centrifuge tubes at -20°C until DNA purification was conducted. Once all measurements were logged, we clipped ventral scales to track any repeat snakes. Snakes from the surrounding areas in the same ecoregion were sampled in the same manner. These snakes were in the same county or surrounding counties of the zoo.

Detection of O. ophidiicola. — For DNA extraction, 400 µL of lyticase (300 U/µL) was added to each tube and incubated at room temperature for 24 hours to digest cellular membranes. Following incubation, samples were centrifuged at maximum speed (approx. 18,000 rpm) for 1 hour to maximize DNA and particulate recovery. We extracted DNA using a Qiagen Blood and Tissue Kit (Qiagen CAT# 69506).

After purification, DNA concentrations were assessed using Nanodrop (ThermoScientific NanoOne[C]). Samples containing detectable genetic material were screened for *Ophidiomyces ophidiicola* using qualitative PCR (qPCR) (Allender et al., 2015). Each sample was run in triplicate, and results were considered valid only if at least two out of three replicates met the established protocol criteria.

Statistical Analysis. — All statistical analyses were conducted in R Studio (Team,2023). Chi-square tests were used to examine the relationship between infection status and species. Logistic regression was performed to assess whether body condition index (BCI) and mass influenced infection probability. An independent t-test compared BCI between infected and non-infected individuals. A linear regression model was used to determine whether infection status significantly predicted BCI. Normality of continuous variables was assessed using the Shapiro-Wilk test. For non-normally distributed data, appropriate transformations or non-parametric methods were considered. Statistical significance was set at $\alpha = 0.05$. All figures were generated using the ggplot2 package (Wickham,2016).

<u>RESULTS</u>

Infection Rates. — We first compared infection prevalence between wild, and zoo-caught populations. The wild caught snakes (N=33) had 4 infection-positive, whereas the zoo caught snakes (N=112) had 16 infection-positive. Infection rates did not differ significantly between wild (12.1%) and zoo (14.3%) snakes ($\chi^2_1 = 0.13$, $p = 0.72$; Fisher's exact P= *0.60)* (Table 1). A logistic regression model (infection v. origin) confirmed this as well (P=0.75). Having established that infection prevalence did not differ we next examined whether body condition varied between the two groups.

Body Condition Index (BCI) Comparison. —Next, we compared the body condition index (BCI) between the wild and zoo-caught snakes. BCI was computed for each snake that had the correct data. Wild snakes (n-25) had a mean BCI of 0.0299, while the zoo-caught snakes (n-101) had a higher mean BCI of 0.0378. The median BCI in wild snakes was 0.0193, compared to the median of 0.0404 in zoo-caught snakes. These summary statistics suggest that, on average, the zoo-caught snakes were in better body condition (heavier for their length) than the wild-caught ones (Table 2).

Before formal testing, we assessed the distribution of BCI. BCI values were right skewed in both groups (Shapiro-Wilk normality test $p<0.001$ for each), so a non-parametric test was chosen for a robust comparison. A Mann-Whitney U test indicated that the zoo caught snakes have a significantly higher BCI than wild caught snakes (U-912, P=0.032). In contrast, a Welch's t-test gave P=0.053, just above the significant threshold, reflecting the fact that the means are somewhat different, but variability is high. This was consistent when evaluated with a one-way ANOVA (BCI by group) yielded P=0.051. Despite the borderline parametric result, the non-parametric test confirms a significant difference. It suggests that snakes caught on zoo property (protected, consistent food source) tend to carry more body mass for given length, whereas wild snakes show lower BCI on average.

Species Composition Differences. —Finally, we compared the species composition of the snake populations between the wild and zoo datasets. A total of 16 snake species were represented across both datasets (Table 2). The wild snakes sampled included 14 species, where the zoo-caught included only 10 species. Several snake species present in the wild populations were entirely absent from the zoo collections (e.g. *Farancia abacura* and *Nerodia rhombifer*). Conversely, the zoo population had species not observed in the wild caught snakes (*Coluber constrictor* and *Opheodrys aestivus*). Moreover, the relative abundances of species differed greatly between the groups. For instance, *Pantherophis obsoletus* (Texas rat snake) was the dominant species in the zoo population (48 out of 112 snakes, ~43%), whereas it constituted a much smaller fraction of the wild sample (4 out of 33, ~12%). Similarly, *Nerodia erythrogaster* (plain-bellied watersnakes) was common in both but proportionally higher in the zoo (19.6% vs 18.2% of each sample). In contrast, species like *Storeria dekayi* (Texas brown snakes) and *Agkistrodon piscivorus* (cottonmouths) made up a larger share of the wild sample relative to the zoo (Table 2). Species composition is notably different between wild and zoo samples. The zoo population is largely composed of a few species (notably *P. obsoletus*), whereas the wild population includes a broader range of species.

Discussion

This study provides a comparative assessment of ophidiomycosis (SFD) prevalence, body condition, and species composition in snakes from zoological and surrounding wild populations. Contrary to our initial hypothesis, infection prevalence did not significantly differ between zoo and wild caught snakes. This suggests that the presence of *Ophidiomyces ophidiicola* is pervasive and that each population are equally susceptible or exposed to this pathogen. This finding underscores the complex ecology of SFD and suggests that transmission may be occurring both within and beyond managed settings.

While infection rates were similar, zoo-caught snakes exhibited significantly higher body condition indices compared to their wild counterparts. This result likely reflects the more consistent and resource-rich environment provided by the zoo, where wild snakes may have access to stable food sources and reduced environmental stressors. In contrast, wild snakes are subject to seasonal variability in prey availability, predation risk, and habitat disturbance, all of which could contribute to lower body mass relative to length.

A significant divergence was observed in species composition. Zoo-collected snakes were dominated by a few common species, particularly *Pantherophis obsoletus*, while the wild sample encompassed a broader range of native species, many of which were entirely absent from the zoo dataset. This discrepancy likely reflects both the accessibility of certain species to zoo staff and the ecological differences in habitats surrounding the zoo.

The underrepresentation of species such as *Farancia abacura* and *Nerodia rhombifer* in the zoo sample may point to either behavioral avoidance of zoo grounds by these taxa or to sampling biases favoring large-bodied or diurnally active snakes.

Despite the novel insights provided by this study, several limitations should be acknowledged when interpreting our results. One notable constraint is the disparity in sample sizes between zoo-caught (n=112) and wild-caught (n= 33) snakes. This imbalance may reduce statistical power and influence the detection of differences, particularly in infection prevalence. Additionally, the methods of snake detection and capture may have introduced sampling biases. Situations like zoo personnel being more likely to encounter larger-bodied or more behaviorally conspicuous species, which could skew the species composition observed on the zoo grounds. Conversely, some species common in the surrounding wild habitats may be underrepresented due to limited access or avoidance of zoo infrastructure. These factors warrant caution in generalizing the comparative results of species richness and infection prevalence.

These differences in species diversity and BCI could have implications for pathogen dynamics. The skewed species representation in the zoo population may influence community-level resistance or susceptibility to infection, particularly if certain species serve as reservoirs or are more prone to severe disease outcomes. Understanding these dynamics is essential for designing effective conservation and disease mitigation strategies, born within zoological institutions and in surrounding natural habitats.

Although zoos implement quarantine and testing protocols for incoming animals, less attention is typically given to free-ranging wildlife that enters enclosures or traverse's zoo campuses. Our findings suggest that such movements, particularly by snakes with higher BCI and potential subclinical infections, could pose a transmission risk to captive reptile populations. Therefore, zoo staff who are often responsible for capturing and relocating these animals, may need to implement stricter sanitation protocols to prevent the potential transfer of *O. ophidiicola* via contaminated handling tools or footwear.

Finally, this research underscores the potential role of habitat quality and prey stability in shaping disease outcomes. Conservation strategies may benefit from prioritizing vulnerable populations in natural, fragmented landscapes where environmental stressors are high, while also maintaining biosecurity in semi-managed habitats. An interdisciplinary approach that integrates disease ecology, habitat management, and zoo biosecurity could improve our ability to mitigate SFD and other emerging wildlife disease across landscapes.

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LITERATURE CITED

ALLENDER, M. C., M. DRESLIK, S. WYLIE, C. PHILLIPS, D. B. WYLIE, C. MADDOX, M. A. DELANEY, AND M. J. KINSEL. 2011. Chrysosporium sp. infection in eastern massasauga rattlesnakes. Emerg Infect Dis Italic 17:2383-4

ALLENDER, M. C., D. B. RAUDABAUGH, F. H. GLEASON, AND A. N. MILLER. 2015. The natural history, ecology, and epidemiology of Ophidiomyces ophiodiicola and its potential impact on free-ranging snake populations. Fungal Ecology Italic 17:187-196

ALLENDER, M. C., M. J. RAVESI, E. HAYNES, E. OSPINA, C. PETERSEN, C. A. PHILLIPS, AND R. LOVICH. 2020. Ophidiomycosis, an emerging fungal disease of snakes: Targeted surveillance on military lands and detection in the western US and Puerto Rico. PLoS One Italic 15:e0240415

BLEHERT, D. S., A. C. HICKS, M. BEHR, C. U. METEYER, B. M. BERLOWSKI-ZIER, E. L. BUCKLES, J. T. COLEMAN, S. R. DARLING, A. GARGAS, R. NIVER, J. C. OKONIEWSKI, R. J. RUDD, AND W. B. STONE. 2009. Bat white-nose syndrome: an emerging fungal pathogen? Science Italic 323:227

FISHER, M. C., D. A. HENK, C. J. BRIGGS, J. S. BROWNSTEIN, L. C. MADOFF, S. L. MCCRAW, AND S. J. GURR. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature Italic 484:186-94

LIZARRAGA, A. J., L. HART, R. M. WRIGHT, L. R. WILLIAMS, AND J. S. GLAVY. 2023. Incidents of snake fungal disease caused by the fungal pathogen Ophidiomyces ophidiicola in Texas. Front Fungal Biol Italic 4:1064939

RAJEEV, S., D. A. SUTTON, B. L. WICKES, D. L. MILLER, D. GIRI, M. VAN METER, E. H. THOMPSON, M. G. RINALDI, A. M. ROMANELLI, J. F. CANO, AND J. GUARRO. 2009. Isolation and characterization of a new fungal species, Chrysosporium ophiodiicola, from a mycotic granuloma of a black rat snake (Elaphe obsoleta obsoleta). J Clin Microbiol Italic 47:1264-8

TEAM, R. C. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

WICKHAM, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

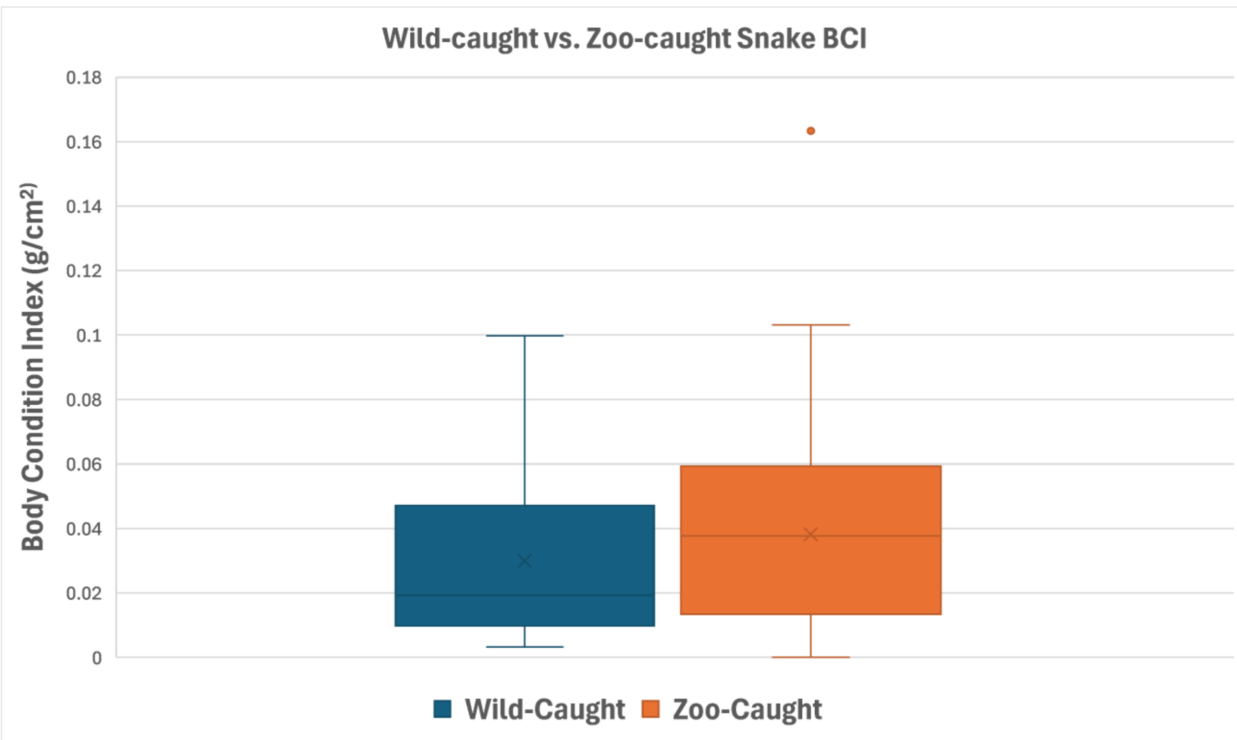

Fig. 2. Box and whisker plots of BCI for wild-caught vs zoo-caught snakes.

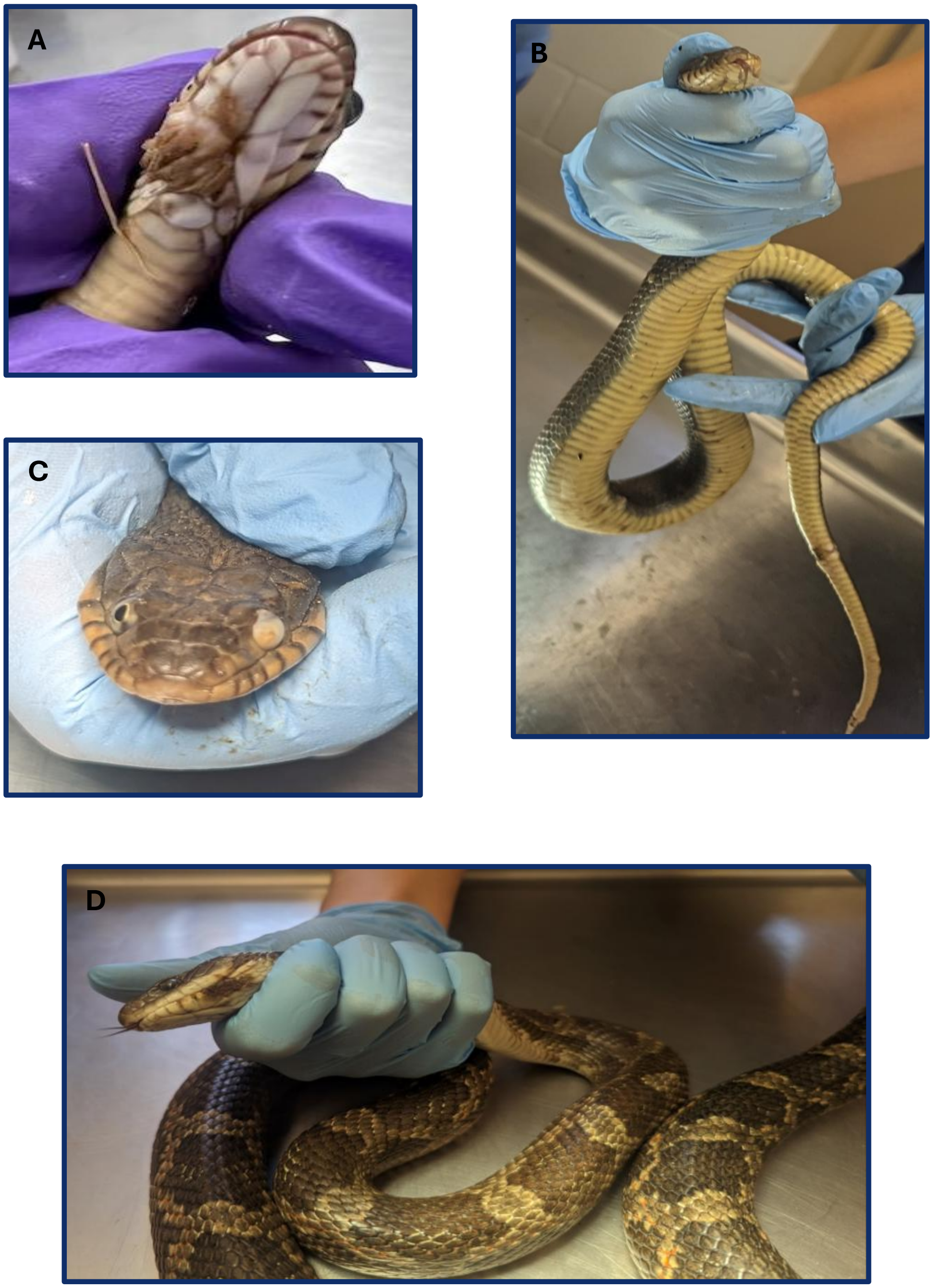

Fig. 1. Images of lesions on wild snakes captured on the zoo campus that tested positive for Ophidiomyces ophidiicola. (A) Lesion on chin of a Texas rat snake (*Pantherophis obsoletus*); (B) clouding of the left eye in a Plain-bellied watersnake (*Nerodia erythrogaster*) with fungal infection; (C) necrotic scales and lesions along the ventral body of another Plain-belled watersnake. (D) lesions on mouth of Texas rat snake that is negative for O. ophidiicola

Table 1. Infection prevalence (proportion of snakes positive for *Ophidiomyces ophidiicola*) in wild vs. zoo caught snakes in eastern Texas

Group	**Total Snakes (N)**	**Infected (n)**	**Infection Prevalence (%)**
Wild Populations (regional)	33	4	12.1
Zoo Population (Texas)	112	16	14.3

Table 2. Frequencies of snake species observed in the wild sample (n= 33) versus the zoo-campus sample (n = 112).

Species	**Wild Count (N=33)**	**Zoo Count (N=112)**
Pantherophis obsoletus	4	48
Nerodia erythrogaster	6	22
Agkistrodon contortrix	2	13
Thamnophis proximus	4	9
Agkistrodon piscivorus	4	8
Nerodia fasciata	1	6
Storeria dekayi	4	3
Masticophis flagellum	1	1
Thamnophis marcianus	2	0
Coluber constrictor	0	1
Farancia abacura	1	0
Hypsiglena jani	1	0
Nerodia rhombifer	1	0
Opheodrys aestivus	0	1

Pantherophis emoryi	1	0
Virginia valeriae	1	0