REPTILES & AMPHIBIANS

Ophidiomycosis in a Timber Rattlesnake (*Crotalus horridus* Linnaeus 1758) with Behavioral and Thermal Observations: A Case Report from Georgia, USA

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Ophidiomycosis (also known as Snake Fungal Disease or SFD) is caused by *Ophidiomyces ophiodiicola* (Oo; Sigler et al. 2013). When first described by Rajeev et al. (2009) as *Chrysosporium ophiodiicola*, it was isolated from a captive Black Ratsnake (*Elaphe obsoleta obsoleta,* now *Pantherophis alleghaniensis*). Oo infections are now known to be widespread across North America and affect snakes from the families Viperidae, Colubridae (*sensu lato*), and Elapidae (Allender et al. 2020; Lorch et al. 2016), but the true extent of susceptible species is unknown. In Georgia, Oo infections are known in both wild and captive populations (Haynes et al. 2020; Patterson et al. 2021). Herein we report ophidiomycosis in an adult male Timber Rattlesnake (*Crotalus horridus*) (SVL = 1,340 mm, mass = 1,700 g) from the Cedar Creek

Figure 1. Post-mortem views of the head (left) and tail (right) of an adult male Timber Rattlesnake (*Crotalus horridus*) showing skin lesions and necrosis of the skin around the vent. Photographs by Anna Tipton.

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Wildlife Management Area (WMA) in Central Georgia that was included in a radio telemetry study since 2020 (Tipton et al. 2023). Snakes in this population also have been monitored since 2021 for Oo.

Snakes affected by ophidiomycosis can exhibit behavioral, gross, and histological signs of disease (Paré and Sigler 2016). This Timber Rattlesnake exhibited all three types of signs of disease. The snake, originally found on 4 October 2021, exhibited no clinical signs of Oo infection (although it was not swabbed for testing at that time). A radio transmitter was implanted surgically on 5 October 2021 and the individual was released at the original site of capture on 8 October 2021. The snake was observed subsequently via radio telemetry once or twice weekly until 15 April 2022, when it was found dead in the field with gross signs consistent with ophidiomycosis. Below, we outline behavioral observations of this individual and contrast them with a co-occurring conspecific that did not exhibit any clinical signs of ophidiomycosis during the same period.

Before the winter inactive period (8 October to 23 November 2021), this adult male moved 0–158 m (mean =18.6 m/day) between successive relocations. Seven additional adult male Timber Rattlesnakes that were monitored during the same period moved 12.8–38.9 m/day (mean ± SE = 19.1 ± 3.4 m/day). Noticeably abnormal behavior did not commence until the winter period, when the focal individual frequently was observed basking. From 17 December 2021 to 12 April 2022, it was seen on the surface at 12 of 13 relocations. During this same period, body temperatures (recorded from a temperature-sensitive radio-transmitter pulse rate) at times of relocation were 25.1–36.5 °C (mean ± SD = 32.5 ± 3.9 °C). During six consecutive relocations from 24 February to 1 April 2022, body temperatures were 33.8–36.5 °C (mean ± SD = 35.4 ± 1.0 °C), likely approaching the critical thermal maximum (Cowles 1939; Stewart 1965). The snake was overwintering at a large communal hibernaculum and also was observed basking on days with cooler temperatures. At 1248 h on 14 January 2022, for example, it was seen basking outside the hibernaculum at an air temperature of 11.4 °C and ground temperature in direct sunlight where the snake was basking of 18.5 °C. During the same period (17 December 2021 to 8 April 2022), an adult female at the same communal overwintering site (~2 m away in the same rock outcrop) exhibited body temperatures of 12.8–21.0 °C (mean ± SD = 15.0 ± 2.2 °C) and was never observed basking on the surface.

Gross signs of infection first became evident on 24 February 2022; these included noticeable lesions and nodules consistent with Oo on the head, tail, and body (Fig. 1, note that images were taken post-mortem). On 3 March 2022, the snake left its overwintering shelter (the earliest recorded egress date among 21 telemetered rattlesnakes on the site in 2022) and moved 238 m to a position along and under a fallen pine log, where it remained on the surface until 12 April 2022. During this timeframe, the snake began ecdysis and rapidly deteriorated in condition, losing weight and unable to complete the normal molt cycle. On 15 April 2022 it was found dead at the same location. At the time of death, the individual had lost 26% (1,700 g to 1,250 g) of its body mass since October 2021. The carcass was frozen and sent for necropsy at the Wildlife Epidemiology Lab, University of Illinois Urbana-Champaign, in September 2022. External gross lesions such as raised crusts consistent with ophidiomycosis were present on the snake's venter. Internal gross lesions were not observed. Tissues were autolyzed and had freeze artifacts, which might have interfered with findings because these can render a sample unsuitable for histological evaluation (hence, no histological images are included in this paper); however, general findings were consistent with an Oo infection, including arthroconidia in the encrusted regions of the skin. The snake was determined to have ophidiomycosis with a fungal load of 3032.75 copies/ng of DNA (the molecular methods used to support the diagnosis can be found in Allender et al. 2015).

Ophidiomycosis can cause behavioral changes in wild populations of snakes, including Pigmy Rattlesnakes (*Sistrurus miliarius*; Lind et al. 2023), Eastern Massassauga Rattlesnakes (*Sistrurus catenatus*; Tetzlaff et al. 2017), and Timber Rattlesnakes (McBride et al. 2015; Lorch et al. 2016). Some snakes exhibited what could be termed behavioral fever (see Burns et al. 1996) by basking outside winter hibernacula (e.g., Clark et al. 2011; Tetzlaff et al. 2017; Lind et al. 2023), which is consistent with our observations.

We documented a 26% decline in the focal individual's body mass during the overwintering period and abnormal ecdysis at the time of death. Snakes affected by ophidiomycosis are known to undergo ecdysis more frequently than unaffected snakes (see Lind et al. 2023 and references therein). This could be indicative of costs associated with Oo infections, especially in larger-bodied snakes, although further studies are necessary before causation can be positively ascertained.

This case clearly demonstrates an association between ophidiomycosis and abnormal behavior in a Timber Rattlesnake. However, in the absence of an Oo swab prior to the winter behavioral observations, we cannot state whether the severe infection emerged as a result of the abnormal behavior or *vice versa*. Also, given that this individual died during ecdysis, more targeted field and experimental research could evaluate costs associated with increased frequency or abnormal timing of ecdysis and possible links between Oo infection and overwintering dynamics, including winter basking frequencies and the use of communal or solitary hibernacula.

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