

## Investigating the Impact of Group Holding on the Transfer of *Ophidiomyces ophidiicola* DNA in Free-ranging Lake Erie Watersnakes (*Nerodia sipedon insularum*)

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**ABSTRACT:** Ophidiomycosis threatens snakes worldwide. We swabbed free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) for quantitative PCR detection of *Ophidiomyces ophidiicola* before and after group and individual holding in pillowcases. Our results indicate that group, rather than individual, holding does not significantly increase detection of *O. ophidiicola* DNA.

Ophidiomycosis, caused by *Ophidiomyces ophidiicola* (Allender et al. 2015a), has been reported in numerous snake species and commonly presents as crusts and necrotic scales (Lorch et al. 2016; Baker et al. 2019). Transmission is hypothesized to involve contact between individuals (Lorch et al. 2016; McKenzie et al. 2020). Ophidiomycosis was first reported in Lake Erie watersnakes (LEWS; *Nerodia sipedon insularum*) in 2009 (Lorch et al. 2016) and hundreds of individuals are captured with minimal biosecurity during annual population monitoring. Our objective was to determine the effect of holding methods on *O. ophidiicola* detection in LEWS. We hypothesized that snakes kept in group pillowcases would show increased *O. ophidiicola* DNA quantity compared with snakes kept in individual pillowcases.

Snakes ( $n=89$ ) were captured by hand at three sites (Gibraltar Island, Kelleys Island State Park, and Middle Bass Island State Park) in Lake Erie, Ohio, US, with hands being covered by clean nitrile gloves or cleaned with alcohol-based hand sanitizer before each capture. Each snake was secured in its own clean transport pillowcase before sampling. Pillowcases were not tested with quantitative (q)PCR but were washed with diluted bleach before each use based on

previous disinfection work (Rzadkowska et al. 2017). Snakes were visually inspected by two examiners (K.D. and E.H.) for lesions suggestive of ophidiomycosis (Baker et al. 2019). A full-body swab (S1) was collected from each snake by passing a cotton-tipped applicator along all surfaces of the body. Snakes were then placed in clean group or individual pillowcases in an alternating fashion, for a total of 15 snakes in each site-specific group pillowcase. Snakes remained in these exposure pillowcases for at least 30 min before a second full-body swab (S2) was collected. Swabs were placed in individual, sterile, 2-mL Eppendorf tubes and frozen at  $-20$  C until processing. Snakes were released at their original capture location. All work was approved by The Ohio State University's Institutional Animal Care and Use Committee (protocol no. 2013A00000106-R2).

We extracted DNA from swabs and performed qPCR for *O. ophidiicola* detection (Allender et al. 2015b). The quantity of DNA (measured in nanograms per microliter) and quality (absorbance at 260:280 nm) were measured using spectrophotometry (Nanodrop, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples were considered positive if the three replicates had a mean cycle threshold value less than the lowest detected standard dilution. Mean fungal quantities (copies per reaction) were standardized to the total quantity of DNA (copies per nanogram of DNA).

Standardized fungal copy number was assessed for normality using the Shapiro-Wilk test; descriptive statistics were tabulated for snakes in group and individual pillowcases, by

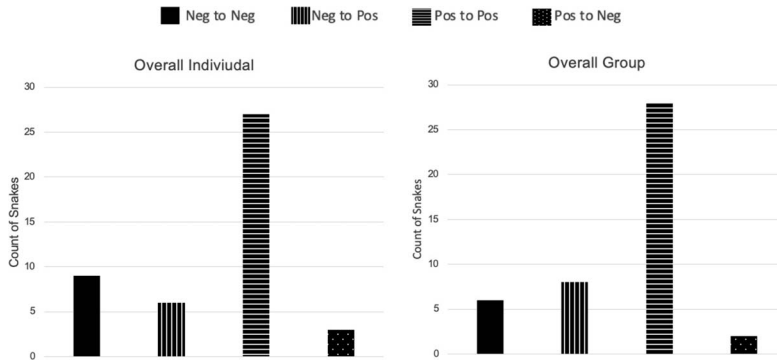


FIGURE 1. Bar graphs showing the number of free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) from three islands in Lake Erie, Ohio, USA, in each of the four categories based on change in quantitative PCR (qPCR) status between initial and postholding swabs of snakes held in either group or individual pillowcases. Neg=negative; Pos=positive.

site and overall. The difference in standardized fungal quantity was calculated between S1 and S2 for each snake. Wilcoxon rank sum tests were performed to compare that difference between animals from group and individual pillowcases within and among sites. To assess whether snakes with a negative S1 were more likely to be positive on S2 if kept in the group pillowcase, each animal was categorized based on S1 to S2 qPCR status: 1) negative to negative, 2) negative to positive, 3) positive to positive, and 4) positive to negative. The prevalence of each category with corresponding 95% confidence intervals (CI) and odds ratios were calculated for animals from individual and group pillowcases at each site and overall. All statistical analyses were conducted using R software, version 3.5.2 (R Development Core Team 2016); statistical significance was assessed at  $\alpha=0.05$ .

Lesions consistent with ophidiomycosis were detected in 74/89 LEWS. Of the snakes with lesions, 56/74 (76%) were qPCR positive on S1. Overall, 67% of LEWS were initially qPCR positive; 61% were qPCR positive on both S1 and S2 (the Supplementary Table). Of snakes in group pillowcases, 18.2% (95% CI, 8.2–32.7%) converted from negative to positive, whereas 13.3% (95% CI, 5.1–26.1%) of snakes in individual pillowcases also converted from negative to positive (the Supplementary Table and Fig. 1). Snakes that were negative on S1 had no significantly greater odds of

testing positive on S2 after being in the group pillowcase compared with those in individual pillowcases. There were no differences in detection among sites.

Our finding that eight initially negative snakes became positive after group holding suggests the possibility of horizontal transfer of *O. ophidiicola*, as recently described by McKenzie et al. (2020). We also documented that six individually housed snakes converted from negative to positive. False-negatives have been reported in the diagnosis of this pathogen but are decreased with thorough swabbing techniques (Hileman et al. 2018), as used in our study. Fungal quantity trended lower on S2, compared with S1 (Fig. 2), with 58% of individuals having a decrease in fungal quantity. This may be due to physical removal of fungus through swabbing or through contact with other snakes or the pillowcase. It is unclear why some snakes from both individual and group pillowcases decreased in fungal quantity between S1 and S2 and others increased.

The high prevalence of qPCR-positive animals decreased this study's ability to evaluate changes in detection of *Ophidiomyces* DNA. Based on the observed proportions of snakes converting from negative to positive in group pillowcases (0.18) compared with individual pillowcases (0.13), the power of our study to detect a statistically significant difference was 0.08. To achieve a power of

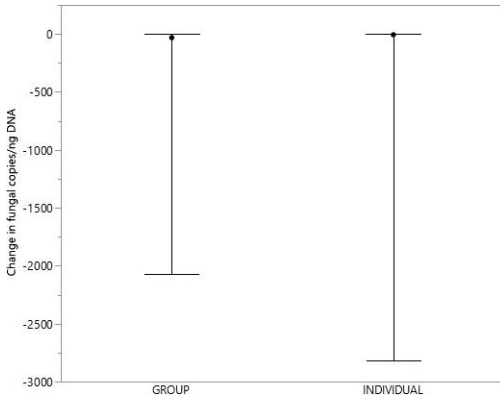


FIGURE 2. Median difference in standardized fungal copies between initial and postholding swabs in free-ranging Lake Erie watersnakes (*Nerodia sipedon insularum*) from three islands in Lake Erie, Ohio, USA, held in group or individual pillowcases. Error bars show the 25th and 75th percentiles.

0.8 with an  $\alpha$  level of 0.05 and our sample size for this study, the difference between the proportions would need to be at least 0.25. Therefore, we recommend repeating this study in a population with a lower pathogen prevalence to achieve a larger effect size or in a similar population with a larger sample size.

Biosecurity is critical when working with wildlife pathogens. Studies have analyzed disinfectant efficacy for specific pathogens (Johnson et al. 2003; Bryan et al. 2009; Rzadkowska et al. 2016), and recent work found that capture method is important in the transmission of amphibian pathogens (Mendez et al. 2008; Gray et al. 2018). Our study found no significant differences with the biosecurity measures implemented but because of its low power and the potential disease transmission ramifications of not using biosecurity, we recommend applying our results to management practices with caution.

#### SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-21-00010>.

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