The Restoration of Two Survey Networks at Hampden-Sydney College to Survey Snakes of Ranavirus

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Abstract

Despite the large impact ranavirus has on ectothermic vertebrates, little is known of its effects on snakes. In June 2021, two old survey networks on the campus of Hampden-Sydney College were restored and reconstructed, specifically near the Wilson Trail and the Observatory. We placed and replaced Artificial Cover Objects such that a total of 50 plywood and 50 tin sheets were placed at each site. In June and July, the sites were checked twice per week, with 48 hours between each survey. Captured snakes were brought to the lab, measured, marked, and tissue sampled for ranavirus. Seventeen snakes were caught this summer, including 16 *Carphophis amoenus* and two *Diadophis punctatus.* All tissue samples are being stored for future genetic testing for ranavirus.

Introduction

Coronavirus has put viruses at the front and center of everyday news because of its direct impact on humans. Perhaps the wildlife equivalent of this is *Ranavirus*, a genus of lethal pathogens known to infect ectothermic vertebrates including fish, amphibians, and reptiles. Ranaviruses infects dozens of species of vertebrates, but the majority of studies have been done on amphibians due to their severe negative impacts in this group [1]. There have been numerous accounts of amphibian mortality events being associated with or directly caused by ranavirus. Research conducted mostly in the last two decades has included genetic characterization of ranavirus found in samples, environmental DNA detection, and histological examination [2].

Although ranavirus can have a significant impact on amphibians, the impact in reptiles are less well-studied. Reptiles are physiologically and ecologically different from amphibians, so it is important that more research be dedicated to the former group. Ranavirus is known to infect 12 different families of reptiles [3]. The impact ranavirus has on reptiles, specifically snakes, is widely unknown due to their secrecy and the lack of studies done on them. Snakes are known to be host species; however we lack information on the different effects of ranavirus between hosts [1].

This summer, we restored an existing survey network in the woods behind the Observatory on campus, and we reconstructed and altered a survey network on the Wilson Trail which was partially destroyed during the construction of the new dorms. Following this, we captured and tissue sampled all non-venomous snakes to test for the presence of ranavirus DNA. We used Artificial Cover Objects (ACO) which act as a shelter for a variety of organisms and are often used to survey terrestrial herpetofauna. ACOs can be used to find snakes with higher probability than other survey methods. Furthermore, using ACOs will prevent obstruction or damage to natural microhabitats where terrestrial herpetofauna reside, if those locations were searched instead of using ACOs [4].

Materials and Methods

Two survey networks were created and used to capture and tissue sample snakes. One site was located behind the Observatory (37°14’13”N, 78°28’14”W; Fig 1) and one site was alongside the Wilson Trail (37°14’36”N, 78°27’58”W), on the campus of Hampden-Sydney College in Prince Edward County, Virginia.

First, we removed the remnants of the previous two ACO networks used in an earlier study [5]. This included all flagging, markings, and ACOs. The survey networks were then recreated to have straighter lines and to create new transect lines at the

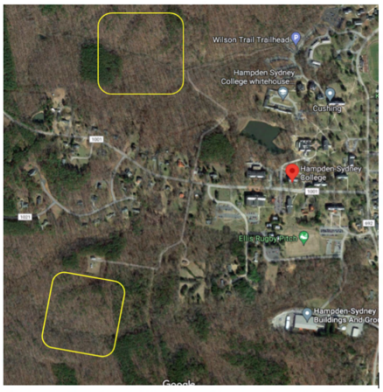

Figure 1. Satellite view of the Wilson Trail and the Observatory survey sites

Wilson Trail site to replace the portion that had been destroyed. One hundred ACOs were placed at each site, 50 tin sheets and 50 plywood boards, each measuring 0.61 x 1.22 meters, placed 20 meters apart on bare soil, after raking leaf litter aside. Some old tins were reused and several of those remained in their original locations from the previous survey. After the networks were completed, the ACOs were given two weeks to settle. The two sites were then surveyed from 06/08/21 and 07/13/21. Both sites were checked two times per week with 48 hours between each check to disrupt the ACO habitat as little as possible.

Snakes were captured using sterile techniques (nitrile gloves and disinfected containers) and brought to the lab for processing using aseptic techniques. Two people were used to sample each snake. One person would take the measurements and samples while the other recorded all the data. This prevented the data sheets and tissue vials from coming in contact with a possibly infected snake. Processing included observing and documenting snakes for any distinct features (injury, infection, or gravidity) and taking measurements (mass in grams and length in cm). Due to the difficulty of measuring, each snake was measured three times for length in order to obtain an average. Next, the snakes were marked by the cautery of two ventral scales using a medical cautery unit in order to prevent recapture [5][6]. All snakes were given an identification number that corresponded with a unique combination of cauterized scales. A 5-mm portion of each snake's tail was removed using a sterile scalpel blade and preserved at -80 °C for later genetic testing. Benzocaine powder was applied to the wound site to slow bleeding and numb the wound. The snakes were then released at the site of capture. As this study progresses, Qiagen DNeasy Blood and Tissue Kits will be used to extract DNA, and PCR will be used to test for ranavirus.

Results

Between The Wilson Trail and Observatory sites, 17 snakes were captured. The Wilson Trail produced 14 total snakes, 11 new and one recapture of *Carphophis amoenus* as well as two new *Diadophis punctatus.* The recaptured *C. amoenus (*ID# 203), was not tissue sampled due to the nubby tail end left over from sampling in a previous year. Another *C. amoenus (ID# 207)* was determined to be gravid and not

Figure 2. Measurements being taken of a caught snake

cauterized to prevent possible damage to the eggs. The Observatory site produced three new *C. amoenus,* all of which were fully processed.

Three of the 17 snakes captured had lesions in the skin, which we suspected were symptoms of Snake Fungal Disease, caused by *Ophidiomyces ophiodiicola*. Two of these were caught at the Wilson Trail and one was caught at the Observatory.

Four snakes were caught under ACOs left from the previous study. Prior to the start of processing snakes in the lab, multiple other snakes were observed during the reconstruction of the networks.

Conclusion

The reconstruction of the ACO networks at both locations provided significant improvements from the previous study. The networks were much easier to follow and survey considering that the lines were straightened and flagging was updated. Furthermore, this allowed us to create updated and detailed mapping to make future surveying more efficient. In fall 2021 and spring 2022 (with a pause from Oct - Feb) we have continued to survey and sample snakes with the goal of obtaining at least 20 individuals per species and ideally 30 to estimate the prevalence of ranavirus in the population. Continued effort is needed because of the secrecy and low rate of captures for snakes.

This summer we captured 17 snakes and samples from 16. The 16 that were tissue sampled were all new, 14 *C. amoenus* and two *D. punctatus*. The tissue samples were stored for future ranavirus testing. The most commonly used quantitative PCR tests for ranavirus detection target portions of the

major capsid protein gene of ranavirus. While effective in all amphibians and some reptiles, this test apparently amplifies part of the snake's genome resulting in a weak false-positive for the virus in these species [7]. Therefore, we will use a less-favored method of conventional PCR and gel electrophoresis to amplify a portion of a DNA polymerase gene in ranavirus (which apparently does not produce false positives in snakes) [8] or attempt to develop a qPCR protocol that works for snakes.

While processing the captured snakes, we noticed that three snakes had a lesion developing on or throughout the body (Fig. 3). All three snakes were *C. amoenus;* two were from The Wilson Trail and one was from the Observatory. We suspect that the lesions were caused by the fungus *Ophidiomyces ophiodiicola.* This is the causative agent of snake fungal disease (SFD), which has been found in multiple Eastern North American snake species. The symptoms include mild to extreme lesions that can be a factor in snake mortality [9]. This fall, in addition to collecting tissue samples for ranavirus, we are also swabbing snakes for SFD.

Figure. 3 Captured snake displaying lesion symptoms

During the reconstruction of the ACO network, multiple species of snakes were observed under or around old ACOs. A *D. punctatus* with a tail clipping believed to be from the previous study was observed. Several other species were observed including *Coluber constrictor, Lampropeltis rhombomaculata, Opheodrys aestivus, and Storeria dekayi.* Unfortunately these snakes were not tissue sampled or measured because they were observed prior to the start of the sampling process. The snakes were found under old tins and plywoods which suggests that ACOs may need to remain in the woods for some time before they are attractive to all snakes.

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Figure 4. Snake found under tin ACO

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