Exploring Growth Conditions for Amanita muscaria

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Abstract

Ranavirus are emerging infectious diseases that have a broad host range. There are studies tying ranavirus to die-off events in amphibians, turtles, and fish, but research on ranavirus in turtles is more limited. We captured two species, *Chrysemys picta picta* (Eastern Painted Turtles) and *Sternotherus odoratus* (Common Musk Turtles), in two water bodies on the campus of Hampden-Sydney College in central Virginia. We captured 103 turtles from 6 June to 23 July and took skin samples to later test for presence of ranavirus using quantitative polymerase chain reaction. By repeating this study in a second year, we will determine the impact of ranavirus on turtle growth and survival.

Introduction

Ranaviruses are a genus of emerging infectious diseases that have a broad host range, infecting amphibians, fish, and reptiles. They are large, double-stranded viruses that can survive in and be transmitted via water as well as in living hosts. Over the last 20 years, more studies of ranaviruses have found them throughout North America and causing mass die-off events in some species (Duffus et al. 2015; Heard et al. 2013). There has been more research on ranavirus in amphibians than in reptiles because amphibians are more easily observed in nature and are more conducive to lab studies. In many amphibian studies, tadpoles are studied which take less time, space, and financing to rear through metamorphosis. Reptiles take longer to mature and require more resources to house, especially as juveniles. In wild populations of amphibians and reptiles, ranaviruses can be asymptomatic or symptomatic and have 0-100% mortality rate in the local population (Price et al. 2013). In species like the Eastern Box Turtle (*Terrapene carolina carolina*) which are listed as vulnerable by the International Union for the Conservation of Nature, surveillance testing is important because ranaviruses may contribute to the decline of the species. Eastern Box Turtles are especially vulnerable to ranavirus perhaps because of their terrestrial and aquatic habits (Johnson et al. 2008).

Ranavirus is present in central Virginian reptiles. Goodman et al. (2013 and 2018) showed that ranavirus DNA was present in local populations of Eastern Box Turtles and Eastern Painted Turtles (*Chrysemys picta picta*). However, these studies only captured a snapshot of the pathogen in the populations because they were only surveyed in one season. In the current study, we are sampling local turtles over two years to see how ranavirus persists and whether it affects the survival and growth of the turtles.

Materials & Methods

The study was conducted at two ponds on the campus of Hampden-Sydney College (HSC) in Prince Edward County, VA: Chalgrove (37°14.5'N, 78°27.8'W) and Tadpole Hole (37°14.7'N, 78°27.2'W). Chalgrove and Tadpole Hole are both approximately 1 ha and located 0.8 km apart. Turtles were collected from 6 June - 23 July 2021. Trapping sites alternated every week until 5 July when we sampled exclusively at Tadpole Hole until 23 July. We trapped at Chalgrove twice with 19 visits and at Tadpole Hole five times with 41 visits. Initially, four traps were set 1-2 m from the shore at a site; from 27 June onwards, six traps were used (Promar collapsible crab/fish traps with dual-ring entrance). Because traps could capture more than one individual at a time, there was a small chance that pathogen transmission could happen among turtles within the traps.

Turtles were removed from the traps and placed in individual plastic containers with air holes to transport to a lab on campus. When collecting and handling turtles, we wore disposable nitrile gloves that were discarded between handling individuals. In the lab, turtles were weighed (to 0.01 g), carapace length, plastron length, and body depth were measured (to 0.1 cm), and each turtle was individually marked using a unique combination of notches filed into their scutes. To sample for ranavirus, we used a 4 mm biopsy punch and disposable scalpel blade to collect a skin sample from beside either one of the hind legs. Tissue samples were then stored at -80 C to later test for presence of ranavirus DNA using a quantitative polymerase chain reaction. The wound site was disinfected with iodine and sealed using VetBond adhesive. The turtles were then transported back to the site and released 5-10 m away from the trap they were captured in. We released all turtles within 24 h of capture except for one that had crawled under a turtle trap and was deceased when we checked the traps. All traps, rubber boots, containers, and a tarp were soaked in a 1% chlorhexidine diacetate (Fort Dodge Nolvasan Solution) for at least one minute (five minutes for traps to penetrate the rope portions) and rinsed with water between use at the different locations.

Results

A total of 103 turtles, including *C. p. picta* (n = 49), *S. odoratus* (n = 52), *Pseudemys concinna* (n = 1), and *Trachemys scripta elegans* (n = 1) were captured. All turtles appeared clinically normal. Both *C. p. picta* and *S. odoratus* were collected from Tadpole Hole and Chalgrove, whereas the *P. concinna* and *T. s. elegans* were captured at Tadpole Hole. Samples were only taken from our target species, *C. p. picta* and *S. odoratus*. At Chalgrove, we captured and tissue sampled 27 *C. p. picta* and 37 *S. odoratus.* At Tadpole Hole, we captured and tissue sampled 21 *C. p. picta* and 14 *S. odoratus;* additionally, we captured one *C. p. picta* that was too small to sample.

Discussion

The first year of this two-year study yielded an adequate sample of individuals from two species (especially when grouping among the two ponds). Having captured around 50 turtles of each species will allow us to have high confidence for prevalence rates, even if ranavirus is rare in the populations. We will replicate our methods in 2022 to collect data that will allow us to compare growth and survival between turtles with and without ranavirus. Even though there were no turtles with visible symptoms, we do expect to have samples test positive for ranavirus. Goodman et al. (2013) found ranavirus in 4 of 23 turtles of *C. p. picta* (17.4%) in Chalgrove and in 6 of 19 turtles of *C. p. picta* (31.6%) in Tadpole Hole despite those turtles showing no disease symptoms. Ranavirus in reptiles and turtles can be nonclinical and still spread within the population, with unknown effects on long-term growth and survival (Duffus et al. 2015). Ranavirus has been researched more extensively in the lab; however, in the wild we have much to learn about the effects of ranavirus over time, particularly in reptiles. Our study will bridge this gap by examining between-year effects of ranavirus in turtles in the wild.

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