Introduction

For the past 20 years, students and researchers at Northern Arizona University (NAU) have been studying and monitoring populations of Northern Mexican and Narrow-headed Gartersnakes in the wild throughout the Southwest and in captivity. Both species were listed as federally threatened by the US Fish and Wildlife Service in 2014, due to substantial population declines (US Fish and Wildlife Service, 2014). In May and June 2014, the 21,227 acre Slide Fire burned through Oak Creek Canyon and surrounding drainages in Coconino County, Arizona, with 46% of the total acreage burning at moderate to high intensities (Nowak, 2018). Post-fire erosion and ash flowing down Oak Creek was expected to severely harm the Gartersnake population through the loss of prey fish (Nowak, 2018).

To help protect this population, NAU, with help from the NAU Green Fund, Phoenix Zoo, US Fish and Wildlife Service, Arizona Game and Fish Department, the US Forest Service, and private donors, established a student-research focused captive research and husbandry program. In 2016, we completed a semi-outdoor vivarium at NAU to house 11 Narrow-headed Gartersnakes collected from Oak Creek after the Slide Fire (Nowak, 2018). Research is focused on studying and understanding Narrow-headed and Northern Mexican Gartersnakes' ecological niches, so that we can better manage and protect these threatened species in the future. Additionally, research is being done to detect the possible presence of *Ophidiomyces ophiodiicola* here in the southwestern U.S., and assist with the development of management strategies that will allow for successful recovery of both gartersnake species.

Snake Background

The Narrow-headed Gartersnake (*Thamnophis rufipunctatus*) is a riparian obligate species which lives in headwater drainages and uses terrestrial habitat for hibernation (US Fish and Wildlife Service, 2014). The Narrow-headed Gartersnake is an under-water ambush predator, feeding mostly on native fish like Desert suckers (*Catostomus clarkii*), Speckled Dace (*Rhinichthys osculus*), and historically, native trout (US Fish and Wildlife Service, 2014). Narrow-headed Gartersnakes also eat non-native trout species like Brown Trout (*Salmo trutta*), and the gartersnakes play an essential ecological role in streams and tributaries as important prey for avian and other predators (Nowak, 2018).

The Northern Mexican Gartersnake (*Thamnophis eques megalops*) is an aquatic species which lives in lower-elevation rivers, marshes, and stock tanks, and also uses terrestrial habitat for hibernation (US Fish and Wildlife Service, 2014). Being an aquatic predator, these snakes mostly prey on native amphibians and fish, including lowland leopard frogs (*Rana yavapaiensis*), New Mexican spadefoot toads (*Spea multiplicata*), true toads, and desert pupfish (*Cyprinodon macularius*) (US Fish and Wildlife Service, 2014). These snakes also eat non-native species like bullfrogs (*Lithobates catesbeianus*) and mosquitofish (*Gambusia affinis*) (Emmons et al., 2014). Although they were historically common in their native ranges, recent drivers like predation and displacement by non-native species have greatly reduced the amount of occupied habitat for both species (US Fish and Wildlife Service, 2014).

Fungus Background

Ophidiomyces ophiodiicola is a contagious and rapidly spreading fungus that has only been found in snake populations (Frankham et al., 2002). It was originally reported in wild populations of snakes in Europe in the 1980's, but after phylogenetic testing of the fungus, it was determined that a new clade had reached New Hampshire in 2006, affecting a population of pitvipers (Clark et al., 2011; Sigler et al., 2013). A 2006 study of Timber rattlesnakes (*Crotalus horridus*) in New Hampshire revealed a sharp decline in population, thought to be partially due to a fungal infection correlated with an abnormally wet May through October (Clark et al., 2011). Today, the disease has been documented in 23 states as well as in one Canadian province (Allender et al., 2015; Frankham et al., 2002; Sigler et al., 2013). Although there has not been a confirmed case of SFD in Arizona or New Mexico, minute traces of *O. ophiodiicola* DNA were detected in a deceased captive Narrow-headed Gartersnake at NAU (Nowak, 2018).

Fungus Ecology

During an *O. ophiodiicola* infection, the pathogen attaches to the epidermis, resulting in an immediate response of swelling. Within days, the infected epidermis dies, leaving a yellow-brown crust characteristic of Snake Fungal Disease (SFD) (Allender et al., 2015; Lorch, 2015). Infected snakes shed their skins more frequently and exhibit irregular behavior like resting in abnormal places or basking later in the winter season, making them more vulnerable to predation (Allender et al., 2015; Lorch et al., 2016). So far, the search for an effective cure has been unsuccessful and rehabilitation requires antibiotics and active health monitoring, making it impractical to execute in wild population (Allender et al., 2015).

Other than *O. ophiodiicola's* DNA sequence and symptoms exhibited by infected snakes, little is known about *O. ophiodiicola*. Current work is being done to better understand its full distribution, transmission, and infection dynamics. One study showed that snakes occupying aquatic environments have a higher possibility of testing positive for *O. ophiodiicola* in the summer and spring (Mckenzie et al., 2018). Another question that currently being examined is the percentage of asymptomatic (i.e., not showing symptoms) snakes acting as hosts in the wild. Although records and information are limited, one study showed that 12% of the 271 sampled individuals tested positive for *O. ophiodiicola*, but did not show any symptoms (Franklinos et al., 2017).

Other Pathogens of Gartersnakes

Bacterial and microscopic mite infections are also common in captive snakes, and both have been detected in captive and wild Narrow-headed Gartersnake populations (Nowak, 2018). The most common mite found in captive populations is the Snake Mite (*Ophionyssus natricis*). The mites feed on the host's blood by lacerating the soft skin between scales (DeNardo and Wozniak, 1997). Since these mites prefer cold and dark locations, infestations are usually observed on snakes recently emerging from hibernation (DeNardo and Wozniak, 1997). Effects of a Snake Mite infestation are dehydration and anemia, which can be fatal (Baker, 1956). In addition to producing severe anemia and dehydration, *O. natricis* has also been implicated in the transmission of several bacterial, protozoal, and filariid pathogens (DeNardo and Wozniak, 1997). In 2016, at two captive Narrow-headed Gartersnakes at NAU (a male and a female) died

while being brought out of hibernation, and during necropsy by the US Geological Survey's National Wildlife Health Center were found to have systematic bacterial infections caused by microscopic mite infestations (Nowak, 2018).

NAU's Research on Pathogens of Federally-listed Gartersnakes

We use visual encounter surveys (VES) and sometimes Gee Exotic fish trap transects to detect Narrow-headed and Northern Mexican gartersnakes in the wild (Nowak, 2018). In addition to weighing, measuring, and individually marking snakes, we record the animals' physical states and any signs of possible infections, and have collected skin swab samples for SFD and mites and, (Figure 1). We also actively monitor and sample our captive population for mites and evidence of SFD infection and *O. ophiodiicola* presence. We are working with NAU and external collaborators to screen these samples. In doing so, we hope to increase understanding of O. *ophiodiicola* distribution, and be better prepared to recover and manage snake populations if this disease is found to be contributing to the decline of Gartersnake populations in northern Arizona and the surrounding regions.

As of April 2019, the NAU vivarium houses four of the original 11 Narrow-headed Gartersnakes that were relocated from Oak Creek. Two females produced three litters totaling 32 offspring in 2017 and 2018. These juveniles are used as research animals, helping us to understand the needs of both captive and wild Narrow-headed Gartersnakes, and improve our husbandry program accordingly. They will not be re-released into the wild until extensive sampling has been done to determine if they may be infected by the presence or prevalence of *O. ophiodiicola*, and a post-release monitoring plan has been approved.

Understanding the fungus's effects on snakes, as well as its possible abundance and distribution in the western US, is crucial to the survival of federally-threatened Gartersnakes and other native snake species. Anthropogenic activities like increasing infrastructure, transportation and recreation are resulting in habitat loss and fragmentation, making these snake populations more susceptible to stochastic events and disease (Frankham et al., 2002). Unchecked, this disease could easily infect fragmented populations. With additional research support and collaboration, we hope to be able to better understand SFD, and be able to assist local and federal managers in protecting and recovering the threatened northern Mexican and Narrow-headed Gartersnake populations.

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