Addressing a Knowledge Gap in *Spilogale* Disease Ecology: Skunk Cranial Worm, *Skrjabingylus chitwoodorum,* in *Spilogale putorius interrupta*

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Abstract - *Spilogale putorius interrupta* (Plains Spotted Skunk) has been documented to host a variety of macroparasites, but the impacts of these infections are largely unknown. We emphasize the importance of incorporating disease-ecology components into ongoing field-research studies by revisiting previously collected data on infection by the metastrongylid nematode *Skrjabingylus chitwoodorum* (Skunk Cranial Worm) in an Arkansas population of Plains Spotted Skunks that was monitored as part of a large-scale field study. Our reevaluation of the infection data suggests estimates of prevalence based on fecal flotations may underestimate true prevalence and that positive infection status may be correlated with smaller home-range size in female Plains Spotted Skunks. We encourage further research to better understand effects of this and other parasites on Spotted Skunk population vital rates and distribution.

Introduction

Spilogale putorius (L.) (Eastern Spotted Skunk) has declined across its range (Gompper and Hackett 2005), and the US Fish and Wildlife Service has been petitioned to list the midwestern subspecies *S. p. interrupta* (Rafinesque) (Plains Spotted Skunk) for protection under the US Endangered Species Act (USFWS 2012). An increasing body of field research is enhancing our understanding of the subspecies' distribution, habitat, and resource requirements (e.g., Gompper 2017; Higdon 2019; Higdon and Gompper 2020a; Lesmeister et al. 2009, 2013), but the disease ecology of Eastern Spotted Skunks remains poorly studied despite a long-standing recognition that knowledge of parasites and pathogens is critical for informed carnivore management (Funk et al. 2001, Knobel et al. 2014, Murray et al. 1999). As such, the lack of detailed consideration of the potential importance of pathogens for management of Eastern Spotted Skunk represents an important knowledge gap. The Eastern Spotted Skunk Cooperative Study Group (ESSCSG) highlighted the need to prioritize disease-ecology research in the Eastern Spotted Skunk Conservation Plan (ESSCSG 2020).

While disease considerations for management of wild mammals often focuses on microparasites, macroparasites can also be problematic for some hosts (e.g., mange-causing mite species in carnivores, pulmonary and filarial nematodes in ungulates).

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For small carnivores, macroparasites may influence host health in a variety of ways (e.g., Olifiers et al. 2015), but such effects are typically underappreciated. While Plains Spotted Skunks host a variety of macroparasites (Lesmeister et al. 2008b), no current field studies aim to understand whether and how parasitism affects hosts. Here, we examine this issue for a particular nematode species: the metastrongylid nematode *Skrjabingylus chitwoodorum* (Skunk Cranial Worm). This species infects the sinuses of *Spilogale* spp., but the individual and population-scale implications of infection remain unknown. Infection by Skunk Cranial Worm can be particularly damaging in *Spilogale* spp., resulting in bulging, lesions, and significant holes in the skull (Fig. 1; Higdon and Gompper 2020b, Kirkland and Kirkland 1983). Based on insights from studies of *Mephitis mephitis* (Schreber) (Striped Skunk), this infection may cause behavioral abnormalities (Goble 1942, Hughes et al. 2018, Lankester and Anderson 1971). Still, little is known on whether and how the parasite affects living Plains Spotted Skunks or other *Spilogale* species.

The objective of this exploratory study was to reexamine a dataset on Plains Spotted Skunks and Skunk Cranial Worms collected from a site in the Ouachita forests of west-central Arkansas (Lesmesiter 2007; Lesmeister et al. 2008b, 2009,

Figure 1. Damage in an Eastern Spotted Skunk skull (Specimen 589250, National Museum of Natural History) caused by Skunk Cranial Worm infection.

2010) to determine the effect of infection by Skunk Cranial Worm on movement, body condition, and survival. We expected that infection may negatively impact *Spilogale* hosts, which would be observable through smaller home ranges, lower body condition index, and increased risk of mortality.

Methods

The study area was located in the Poteau Ranger District (96,755 ha) of the Ouachita National Forest (690,000 ha), a landscape predominantly managed for an open canopy of *Pinus echinata* Mill. (Shortleaf Pine) and an herbaceous understory. Details of the studies that contributed to the data can be found in the source references. In brief, the host data set is comprised of morphometric (Lesmeister 2007), home range (Lesmeister et al. 2009), and mortality (Lesmiester et al. 2010) data derived from $n = 33$ captured and radiocollared Plains Spotted Skunks. We derived the *S. chitwoodorum* data from an endoparasite fecal survey ($n = 90$ fecal samples from 29 individuals; Lesmeister et al 2008b). Samples were collected from known individuals at capture sites or from their den and resting sites (Lesmeister et al. 2008a). Fecal samples were stored in 10% formalin until laboratory analysis. Endoparasites were prepared for identification via standard sugar (specific gravity = 1.33) and zinc sulfate (1.2) centrifugation concentration-flotation techniques. Ova, oocysts, and larvae were examined using compound microscopy to facilitate determination of species based on morphologic characteristics and linear measurements (Lesmeister et al. 2008b).

From these data sets, we used parasitology data from 23 adult Plains Spotted Skunks (12 female, 11 male) and 82 fecal samples to assess possible impacts of infection by Skunk Cranial Worm on movement, body condition, and survival. Of the 23 focal individuals, 26% were positive for Skunk Cranial Worm, and among these positive individuals, 4 females and 2 males were infected (female prevalence = 33%; male prevalence = 18%).

Because nematode ova production is often temporally variable, we first assessed the efficacy of diagnosing infection by Skunk Cranial Worm from fecal samples using fecal-flotation approaches. We limited the dataset to only infected individuals and then contrasted the diagnosis (positive or negative for Skunk Cranial Worm) for each sample from which multiple fecal samples were collected, assuming all individuals from which the feces collected were positive at the time of feces collection. We calculated a false negative rate as the percent of fecal samples that failed to identify Skunk Cranial Worm ova. To account for individuals that may have become infected during the study, we also calculated the false negative rate using only samples collected at or after the first positive test.

To compare body condition between infected and uninfected individuals, we subdivided the data by sex, and for each sex, we averaged body weight and length across capture occurrences for each Plains Spotted Skunk and calculated a body-condition index for each individual as the residual calculated from the linear regression between body mass and length (head and body). We used Wilcoxon rank sum tests to compare infected and uninfected Plains Spotted Skunk body condition.

To evaluate the effect of Skunk Cranial Worm infection on Plains Spotted Skunk movement, we focused on home-range size and used *t*-tests to compare the 95% contours of seasonal utilization distributions for infected and uninfected individuals, subsetting the data by sex because home ranges for males are up to 6.4 times larger than those of females, depending on season (Lesmeister et al. 2009). We excluded spring home ranges for males because they are significantly larger than home ranges for males in other seasons and due to small sample size of infected individuals (n = 1) during that season.

To assess possible impacts of Skunk Cranial Worm on host survival, we used cause-specific mortality data (Lesmeister et al. 2010) and compared cause of death between infected and uninfected individuals using a chi-squared test. While Lesmeister et al. (2010) also contained information on mean annual survival, we did not contrast infected and uninfected individuals for these categories, as the infection status of individuals may have changed over the course of the study.

Results and Discussion

The false negative rate for Skunk Cranial Worm using the fecal flotation method was 73.08%. In other words, the method incorrectly assigned an infected Plains Spotted Skunk as uninfected 73.08% of the time. Accounting for individuals that may have become infected during the study, the false negative rate was 63.16%. While the prevalence rate observed among adults in this Plains Spotted Skunk population (26%) was consistent with a 25% prevalence for this subspecies based on examination of museum specimens (Higdon and Gompper 2020b), it is clear that temporal variability in ova production may result in a significant underestimation of true prevalence for a population. Novel methods for assessment of the infection status of an individual skunk are thus needed. While methods to detect skull damage may offer a partial solution, those animals that are new hosts or have low-intensity infections and may not yet have clear cranial damage may continue to be diagnosed as uninfected if necropsies are not feasible. Further, the threshold at which an infection induces morbidity (i.e., the intensity at which an infection might result in health or behavioral impacts) for an individual skunk is unknown and warrants investigation, further exemplifying the need for novel assessment methods.

Of the 23 adult Plains Spotted Skunks, for both males and females, we found no differences in the body condition indices of infected and uninfected individuals. In this population of Plains Spotted Skunks, higher body condition index was associated with higher survival rates for males, but lower survival rates for female individuals (although the female results may have been confounded by reproductive status; Lesmeister et al. 2010). Nonetheless, at this point there is insufficient data to suggest that *Skrjabingylus* infection affected body condition of individuals studied in the Ouachita National Forest.

For our analyses, we had enough movement data for 7 female and 10 male Plains Spotted Skunks to facilitate analyses of behavior differences of infected and uninfected hosts. There was no difference between the collective summer, autumn, and winter home-range sizes of infected and uninfected males. For females, annual home-range size for infected individuals was significantly smaller than uninfected individuals (Fig. 2; $P = 0.04$). With the caveat that sample sizes were small, false negative rates are likely high, and the original studies were not designed to test for the impact of *Skrjabingylus* on the host, our result suggests that female Plains Spotted Skunk home ranges may be negatively impacted by infection.

We used all 23 Plains Spotted Skunks to examine survival between infected and uninfected individuals. Cause of death was attributed as avian, mammalian, or other, and some individuals were lost due to dispersal or technical issues, while the remaining individuals survived beyond the study (Lesmeister et al. 2010). Among uninfected individuals, 76.47% died due to predation or other causes, and 17.65% were lost. Half of the 6 infected individuals were lost, and 2 died of predation, so we had little evidence that there was a difference in the proportions of Plains Spotted Skunks that experienced each fate ($P = 0.27$). Although we expected that infection by Skunk Cranial Worm may result in abnormal behaviors that indirectly affects survival, the data did not support this prediction, a result that is seen in the mustelid literature for which *Skrjabingylus nasicola* (Leuckart) is the primary species of concern (Heddergott et al. 2016). Field-based studies on *S. putorius* should prioritize necropsies that can assign Skunk Cranial Worm infestation status to the individual.

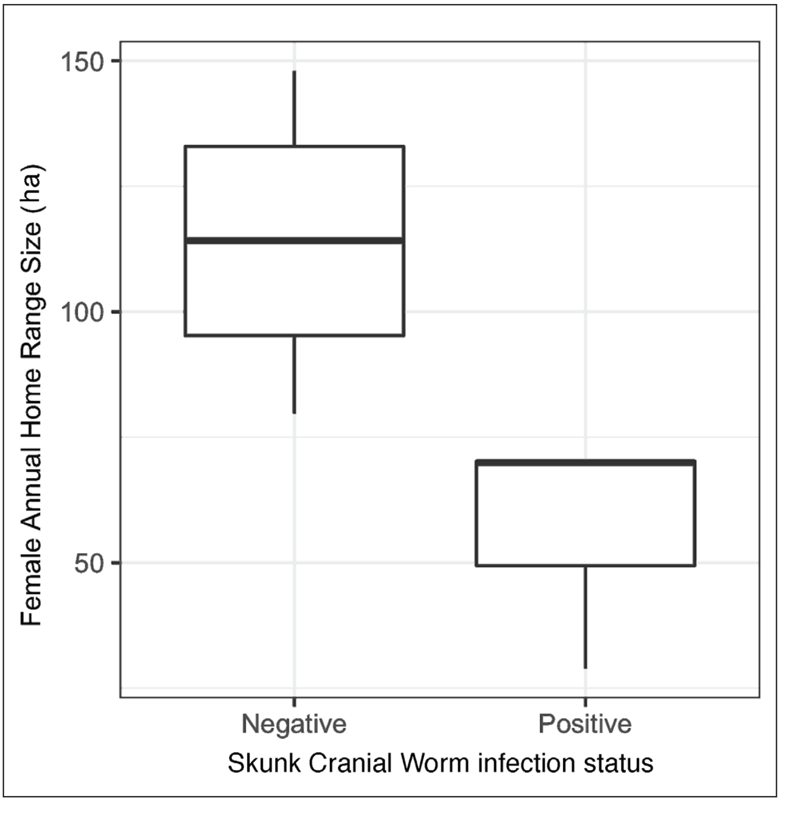

Figure 2. Boxplots of the annual home-range size for female Plains Spotted Skunks subdivided by *Skrjabingylus chitwoodorum* (Skunk Cranial Worm) infection status.

Conclusions and Recommendations

Skunk Cranial Worm is a nematode that results in large holes in the skull of infected individual *Spilogale*. As such, one might expect significant differences in the behavior and fitness of infected and uninfected individuals. While our results identified this for female home-range size, such differences were not observed for other metrics. However, the post-hoc exploratory design of this case study, in combination with sample-size limitations and diagnostic concerns, suggests that the mixed evidence presented herein for an effect of Skunk Cranial Worm on Plains Spotted Skunk should neither be viewed as supportive or rejecting of Skunk Cranial Worm playing an important role in Plains Spotted Skunk health and ecology. Rather, we believe our analyses should be viewed as setting the stage for a more directed study of the topic.

In a broader disease-ecology context, we strongly believe that future studies of Spotted Skunks should be designed to comprehensively examine the impacts of both macro- and microparasites. Most field ecologists have little or no training in ecological parasitology, and as such fail to recognize the potential for putatively non-lethal parasites to have significant direct and indirect impacts at individual or population scales. The initial call for Spotted Skunk disease-ecology work came from the Eastern Spotted Skunk Cooperative Study Group, a collaborative group with 28 member states (https://easternspottedskunk.weebly.com). This group serves as a platform for collaborative efforts across field-based research projects throughout the member states to close the knowledge gap on the disease ecology of Eastern Spotted Skunk. However, doing so will require that the wildlife ecologists conducting studies build stronger relationships with wildlife disease experts trained to diagnose and quantify infection, as well as those who are experienced in incorporating such data into analyses of other topics of management interest.

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