

Introduction

- Roadkill is a common form of human-wildlife conflict. Carcasses can inform wildlife population trends, species distribution, and behavior.
- Researchers can inspect carcasses for evidence of parasites, pathogens, and other health metrics.
- Data obtained from vehicle-killed animals are often limited by non-random sampling and decomposition.

Study System

- Rock squirrels (*Otospermophilus variegatus*) in and around Zion National Park, Utah.
- Ground-dwelling, omnivorous, hibernators, and semi-social.
- Commonly habituate to park tourists.
- Host a variety of parasite species including worms, ectoparasites, and blood parasites



Research Questions

1. How do the traits of vehicle-killed squirrels (sex and activity) affect parasite abundance?
2. Do measures of parasitic infection (richness, abundance, and diversity) differ between roadkill and live-trapped individuals?

Methods

- 19 roadkill and 197 live-trapped squirrels ($n = 216$) from Zion National Park sampled during spring, summer, and fall 2020-2022.
- Roadkill squirrels were frozen until intestines were extracted. Intestines were segmented into small, large, and cecum for microscopic survey at 5x magnification (Platt 2002).
- Fecal samples were obtained from live-caught squirrels and stored in formalin at 4°C. Parasite eggs were extracted using salt flotation and observed under 100x and 400x magnification to determine parasite identity and abundance (eggs/gram of feces).
- Parasite diagnostics were performed to operational taxonomic units (OTUs) using morphological traits.



Figure 1. A. Preparing a study skin and extracting intestines at the Georgia Museum of Natural History. B. Intestines of squirrels measured 2 m on average. C. Fecalizer capsule with feces and saturated salt solution used to identify parasite eggs from live-trapped squirrels.

Results

Roadkill squirrels host more parasite individuals in autumn

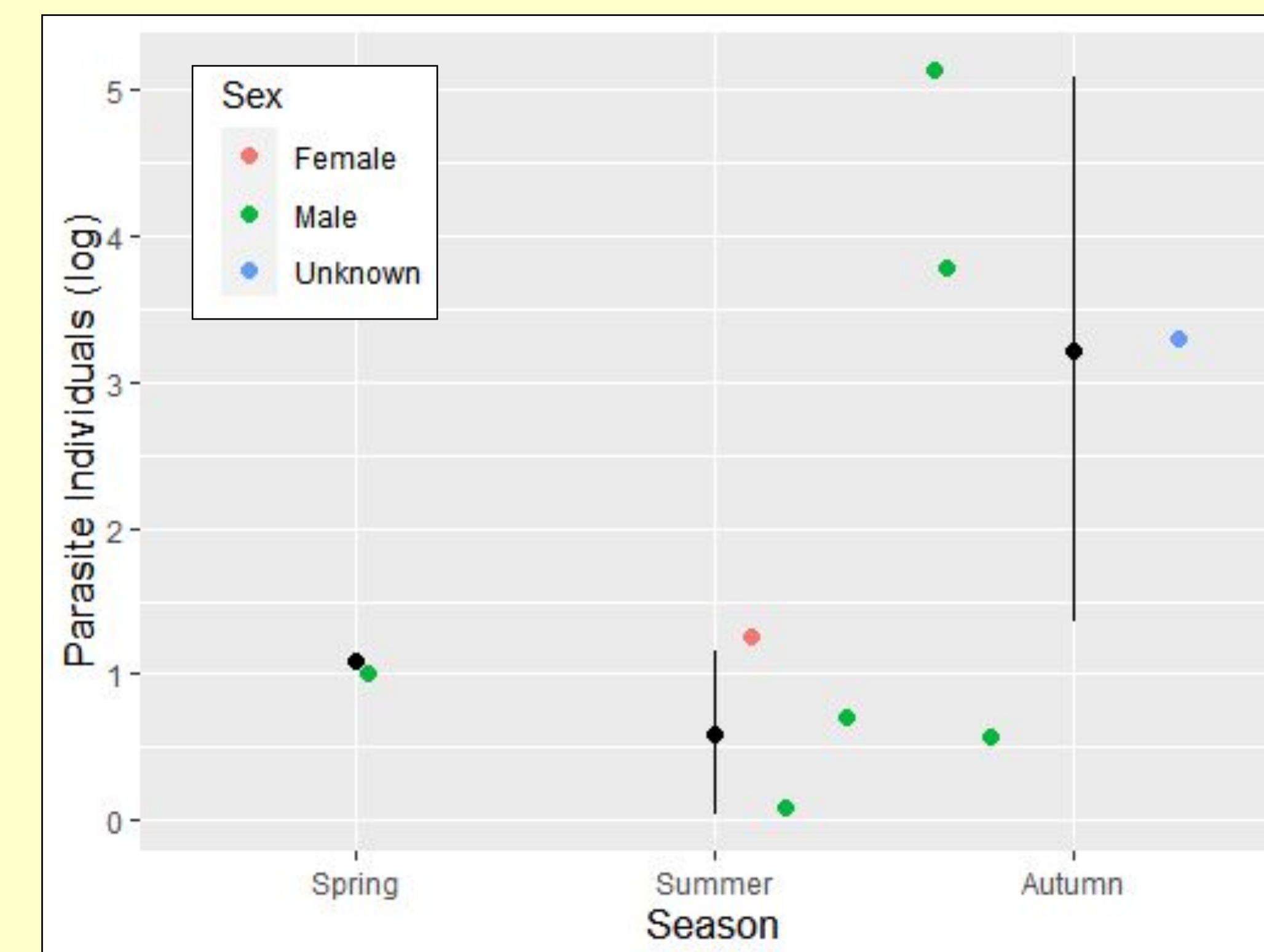


Figure 2. Parasite abundance in roadkill squirrels across seasons. Black dots identify the mean parasite count for each season, black lines show standard deviation (ANOVA test, $F = 3.53$, $p = 0.097$).

- Roadkill squirrels consisted of 11 male, 4 female, 4 unknown
- 73.7% of roadkill contained viable intestines for parasite surveys (14/19)
- Squirrels collected in fall have higher parasite abundance



Figure 3. Example of roadkill not viable for endoparasite survey.

Parasite richness similar for roadkill and live-trapped squirrels

- 6 parasite OTUs identified in roadkill squirrels (abundance 0 - 175).
- 3 parasite OTUs identified in live-trapped squirrels (abundance 0 - 384).

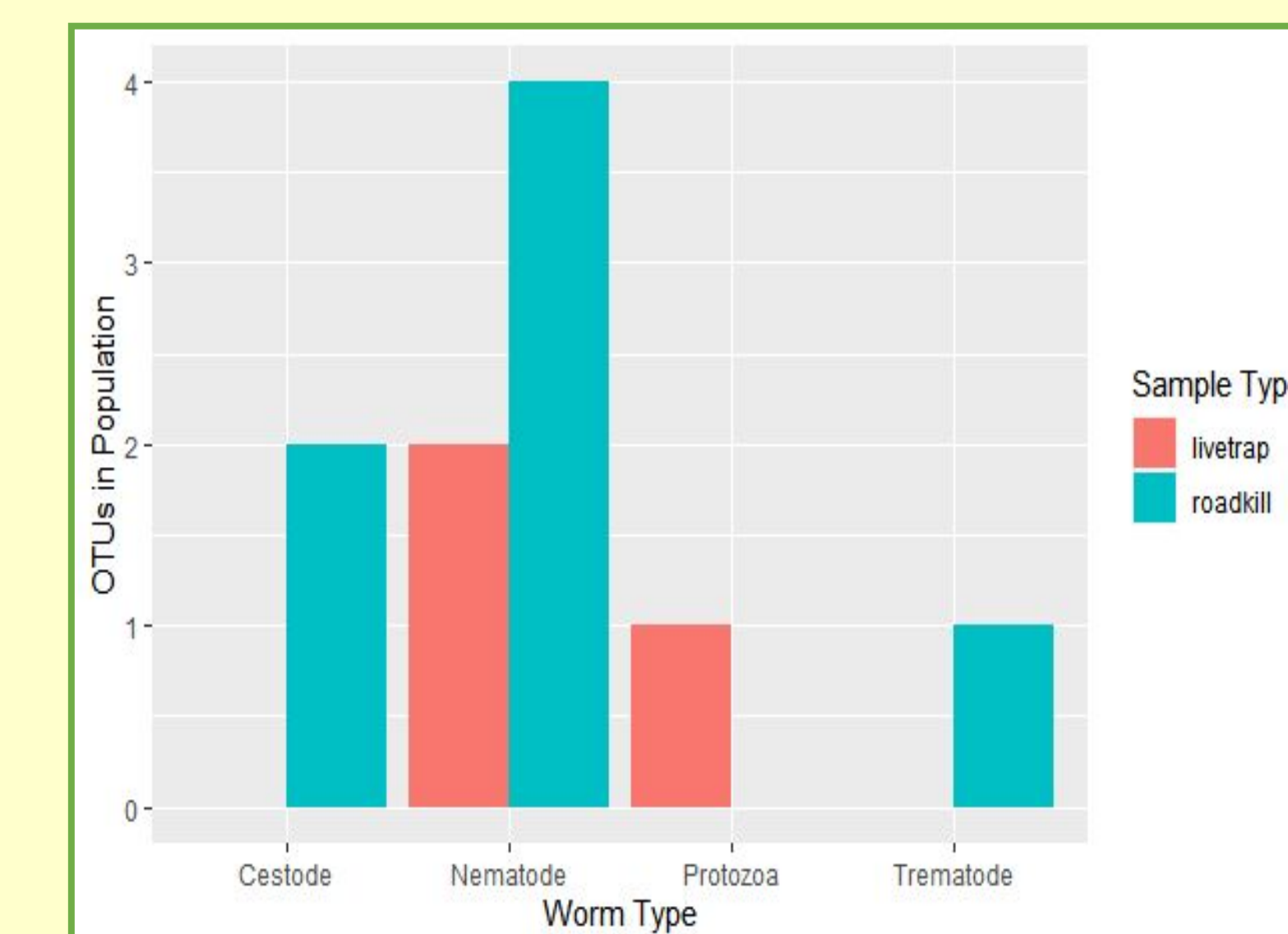


Figure 4. Population level parasite richness between roadkill ($n = 9$) and live-trapped squirrels ($n = 7$) shown by parasite class. Parasite richness is sum of observed taxonomic unit (Anova test, F -value = 3.57, $p = 0.13$).

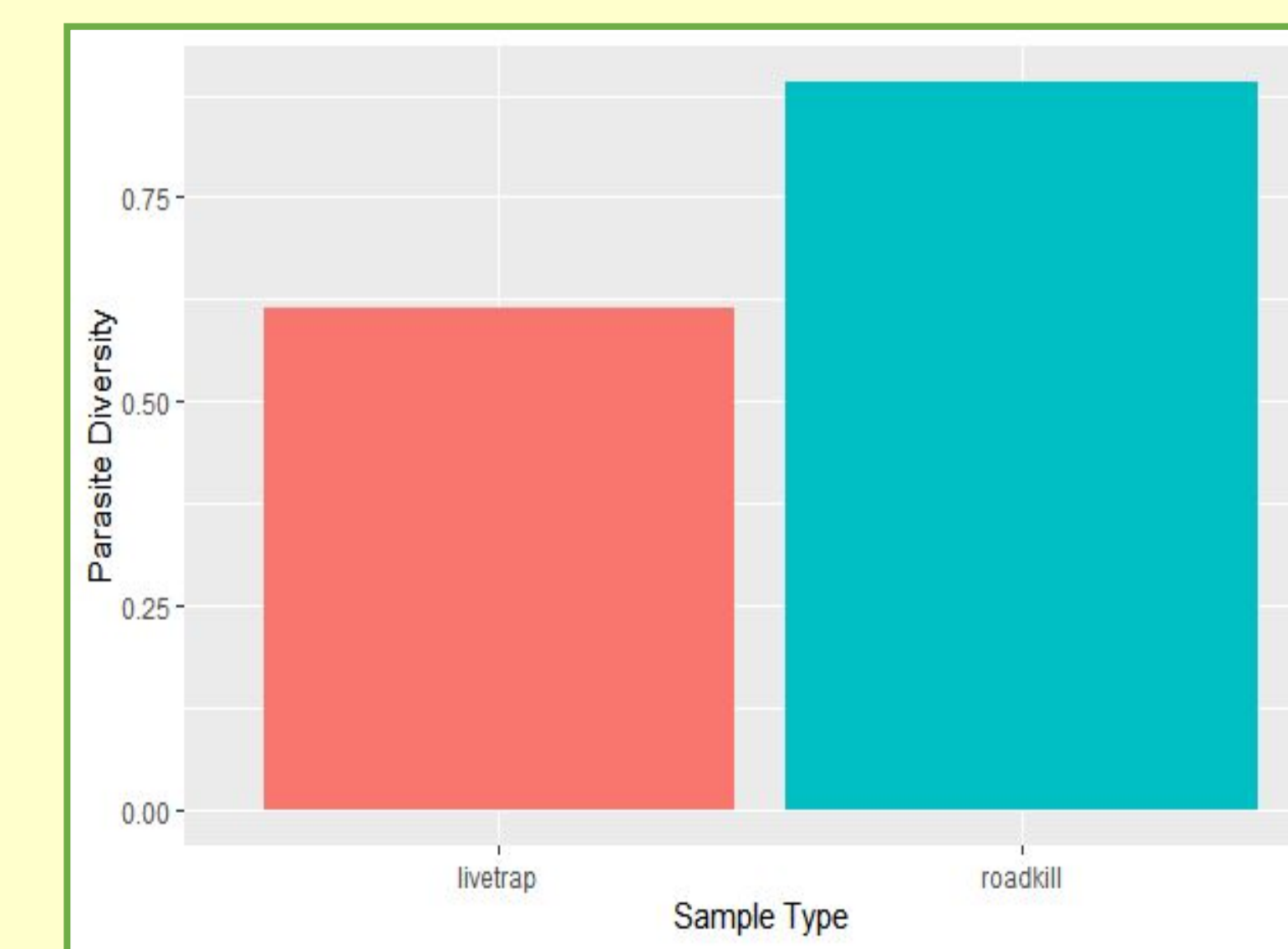


Figure 5. Parasite diversity between roadkill and live-trapped squirrel populations. Diversity value calculated using the Shannon Diversity Index, accounting for parasite abundance and richness.

- OTU richness on the individual scale averaged 1.
- Highest OTU richness in roadkill individual was 3 (2 nematodes, 1 trematode).
- Highest OTU richness in live-trapped individual was 2 (1 nematode, 1 protozoan).



Figure 6. Parasite OTUs identified in roadkill and live-trapped squirrels. A. Adult cestode. B. Adult cestode, worms over 18 cm in length. C. Adult Nematode. D. Adult trematode. E. *Eimeria* eggs in fecal sample. F. *Trichuris* egg. Three nematode OTUs not pictured (one egg and two adult worms).

Discussion

- Male squirrels were killed by vehicles more than female squirrels, likely due to exploratory and dispersal behaviors.
- Parasite abundance is highest in autumn, possibly related to parasite history or peak in population size.
- Parasite richness is higher in roadkill than live-trapped squirrels for three of the four parasite types.
- Intestinal surveys enabled discovery of whole adult worms of either sex, whereas fecal flotations are limited by sporadic egg shedding, and not all parasite types have eggs that float in salt solution.
- A benefit of sampling feces of live-trapped squirrels was that identification was possible to a further OTU than identification of roadkill.
- Due to high abundance of *Eimeria* spp. in live-trapped squirrels, a sampling method that enables detection of microparasites in roadkill samples is needed.
- Parasite diversity metrics were similar for live-trapped and vehicle-killed squirrels. Abundance was higher in live-trapped samples, but fewer OTUs were identified.



Conclusions

- Roadkill can be an effective indicator for parasitic infection in a wild population, provided sampling bias is addressed.
- Measures of infection and parasite diagnostics differ between live-trapped and roadkill samples, but parasite diversity remains similar.
- Our study focused on gastrointestinal parasites, but squirrels would be an excellent study system for bloodborne parasites and ectoparasites, as well.

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