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Effect of a Coevolved Parasitic Nematode on Fitness of the Desert Horned Lizard (Phrynosoma platyrhinos)

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Effect of a Coevolved Parasitic Nematode on Fitness of the Desert Horned Lizard (*Phrynosoma platyrhinos*)

By

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Requirements of the Degree
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Master's Thesis

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Effect of a Coevolved Parasitic Nematode on Fitness of the Desert Horned Lizard (*Phrynosoma platyrhinos*)

A Thesis Presented to
The Faculty of
Western Washington University

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ABSTRACT

Desert horned lizards, *Phrynosoma platyrhinos*, become infested with the gastrointestinal nematode parasite, *Skrjabinoptera phrynosoma*, when the lizards eat the intermediate host of the parasite, harvester ants, *Pogonomyrmex californicus*. This thesis research sought an answer to the integrated question, "How does parasite load affect fitness-relevant performance measures of this sexually dimorphic lizard?" I examined nematode load as a correlate of foraging distance per day, feeding rate, prey choice, home range size, running and endurance, body condition, abundance of non-nematode parasites, hematocrit, and blood immune response in 19 males and 20 female adult desert horned lizards in June-July 2018, in the Alvord Basin (Harney Co., OR) in the northern extreme of the Great Basin Desert scrub.

Foraging-related movement distances and home range size were estimated with repeated sighting locations aided by radio-telemetry. Daily rates of prey capture and comparisons of prey types were accomplished by examination of lizard fecal pellets and pitfall traps for arthropods. Exercise capabilities were measured on a linear racetrack of natural sand substrate; maximum velocity was assessed by high-speed video camera and endurance was measured as distance run before exhaustion. Body condition index was measured as gut-empty body mass divided by snout-vent length. Hematocrit was measured as ratio of packed blood cell volume to total volume of centrifuged blood, and blood immune response was measured as number of leukocytes per 1000 erythrocytes via digital examination of blood slides. Ectoparasitic trombiculid mites were counted by magnified visual inspection. Nematodes were removed by gastric lavage and cloacal lavage with isosmotic saline, then counted and measured. The literature-based estimate of the resting metabolic rate (joules/hr) of nematodes was summed for each lizard and used as the primary metric for nematode load. A variety of supporting data from previous years of on-site field work allowed for more definitive interpretations.

Corrected for body size as a covariate, lizards with a larger nematode load foraged for longer distances, had a heavier body condition, reduced running endurance, and a reduced blood immune

response. Females with high nematode loads consumed fewer of the nematode-infective ant species, *Pogonomyrmex californicus* (POCA). Compared to males, females had a greater snout-vent length, consumed more POCA, had larger fecal pellets, and a greater body condition index. Also, whereas male dietary preference for POCA did not vary with nematode load, female dietary preference for POCA decreased with nematode load and increased with reproductive state. Female fecal size also decreased with nematode load.

Across the range of nematode load observed in summer 2018, nematode load was less detrimental than to the lizards than I hypothesized, with the only statistically significant detriments being in reduced energetic endurance and reduced leucocyte counts. Moreover, lizards with greater nematode loads tended to have increased daily foraging distances and a higher body condition index; correlations possibly due to a compensatory effect, inducing hyperphagia in order to feed both themselves and their parasites. Lizards with the highest of three levels of nematode load demonstrated reduced blood immune response, which could be linked to the immunomodulatory effect of chronic parasite infestation which has been demonstrated in other studies. The statistically insignificant trend of increased prevalence of trombiculid mites in lizards with a high nematode load might be a consequence of more mite encounters as infested lizards move greater distances.

Nematode load may affect dietary preferences of horned lizards. Gravid female lizards consumed primarily POCA. By contrast, males and non-reproductive females consumed comparatively few POCA, instead they ate less available and smaller ants, possible due to their low energy needs and prioritization towards lowered nematode load. I infer that the causes for this putatively stable, coevolved lizard-nematode relationship are 1) that reproductive female horned lizards must eat the large-bodied POCA, the intermediate host of the parasite, to meet their reproductive energy needs, and 2) nematode infestations must have only a modest effect on the survival of their lizard hosts during the energetically challenging reproductive season.

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I would foremost like to thank Dr. Roger Anderson for accepting me as his graduate student, for his support in developing my thesis project and the years of work he has invested to create the field infrastructure needed to complete this project, and for near singlehandedly facilitating travel, survival, and meaningful fieldwork in the remote Alvord Desert Basin, while still finding the time to be jovial and helpful mentor. I would also like to thank Drs. Ben Miner, Deb Donovan, and Jason Ransom for their involvement on my thesis committee and the valuable feedback they have given me through this process.

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Finally, I want to thank anyone involved in the research and conservation of desert environments. Desert ecosystems and the spectacular animals that inhabit them, including the dozen or so species of horned lizards, face increasing threats from anthropomorphic and environmental sources, and I thank anyone who dedicates their time to examining the adaptations that allow these species to inhabit these harsh beautiful environments and how best to protect them for future generations.

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INTRODUCTION

Rationale for research

In recent decades, scientific understanding of parasitism has grown from the study of the pathological effects of parasite on their hosts to an expanding realization of the suite of complex effects which radiate from their hosts to the greater ecological community (Wood & Johnson, 2015). Medically removing coevolved parasites from their ecosystem can substantially alter the natural dynamics of both their hosts and the larger ecological sphere (Hudson, Dobson & Newborn, 1998). The degree of harm a parasite can impose upon their host depends upon factors such as phylogeny, host density, host physiology and host-parasite specificity. Hosts may be less able to resist infestation if parasites are recently introduced (Muldrew, 1953), but hosts existing in a long-coevolutionary relationship with their parasites tend to develop mechanisms of resistance (Wakelin, 1998). Parasite infestations in metabolically energetic hosts in non-nutrient limited environments (*i.e,* mammals and livestock) tend to be highly detrimental (Stein *et. al*, 2002), whereas infestations within smaller ectothermic hosts in nutrient-limited environments can be milder (Eisen, 2016). Moreover, coevolution between parasites and hosts can modify host phenotypes in seemingly beneficial ways, such as facilitating more effective nutrient assimilation (Davis & Proudy, 2019), more adaptable behavioral responses and enhanced resistance to other parasites and pathogens through competition and immunomodulation, both discussed in Weinersmith and Early, 2016. Thus, although parasitism is innately assumed to have some harm, modest levels of infestation by a coevolved parasite can be of little detriment and might have some mitigating effects (Fellous & Salvaudon, 2009).

The two-trophic system of hosts—harvester ants (*Pogonomyrmex californicus*) hereafter referred to as POCA, which are eaten by the desert horned lizard (*Phrynosoma platyrhinos*)—both of which are coevolved with the gastrointestinal nematode (*Skrjabinoptera phrynosoma*) (Lee, 1957), represent a model system for studying parasite-host physiology. Nematodes are universal throughout the horned lizard population, easily accumulating within them because the horned lizards are myrmecophagous, with adult lizards consuming the intermediate host of the nematode *S. phrynosoma,* harvester ants, as a principal component of their diet. Ants pick up desiccated, deceased gravid female nematodes that have been expelled from the lizard's cloaca and transport them and their viable eggs to the colony. Lee (1957) showed that ants which were experimentally fed a dried gravid female worm later presented with nematode larvae housed in membranous cysts within the ant's body cavity. In horned lizards intentionally fed those nematode larval cysts, all lizards became infected and most all larvae developed into a detectable worm. After nematode larvae are transferred to the horned lizards via the ants, they grow within stomach, likely breed within the hindgut, and gravid female worms are expelled with fecal pellets to complete the cycle (Babero & Kay, 2015, Lee, 1957). A survey of desert horned lizards and their parasites in central and eastern Nevada (within the Great Basin Desert) revealed that *S. phrynosoma* is their most common parasite and moderate infestations can yield 100-350 worms; severe infestation more than 1000 worms (Babero & Kay, 2015). In the Alvord Basin in Harney County, Oregon, although these horned lizards also consume as many as 12 other species of ants, the nematode-transmitting harvester ant is their primary dietary choice, at about 60% of their diet (Anderson, 2018). As ant specialists with a specific food acquisition mode (Sherbrooke, 2003, Munger 1983, Pianka 1975) it can be inferred that there is a strong ongoing ecological and evolutionary relationship between desert horned lizards and their ant prey, and therefore between these lizards and their nematodes.

Although there have been a modest number of studies on the behavioral, physiological and population ecology of lizards in the genus *Phrynosoma*, no studies have focused on the how this coevolved gastrointestinal nematode may affect the fitness prospects of horned lizards in the wild. I used a multifaceted approach to explore how much nematode load affects lizard behaviors related to food acquisition, lizard health, and exercise performance. As the inherent costs of sustaining nematode parasites oppose the mild costs of coevolved parasites, how much do we expect these gastrointestinal nematodes to affect horned lizard fitness?

The costs of parasitism

Coevolved parasites, even if widespread in a host population, may have little effect on overall health of their hosts. Consistent with the hypothesis of dampened negative effects over evolutionary time, a lacertid lizards species where 96% of wild adults were infected with an apicomplexan parasite of genus *Hepatazoon* did not show reduced escape distance with greater parasite loads, with researchers attributing the high prevalence of the parasite to its benign nature (Damas-Moreira *et al*. 2014). Similarly, most western fence lizards (*Sceloporus occidentalis*) infected with a coevolved trypanosome did not show reduced foraging success or feeding rates compared to non-infected lizards, but body condition was reduced in infected lizards which were also defending a territory (Eisen, 2016). Furthermore, *Passalus* beetles infected with a nematode parasite demonstrated increased fight strength during male-male competition compared to unparasitized beetles (Vasquez *et al*. 2015).

Conversely, helminth endoparasites such as nematodes which occupy considerable mass within the gastrointestinal tract and act as internal competitors for energy may have substantial detrimental effect on the survival and wellbeing of their hosts. For example, livestock ruminants that were heavily infected with nematodes exhibited decreased food intake and decreased energy output (Holmes, 1994). Also, red grouse infected with their coevolved nematode (*Trichostrongylus tenuis*) consumed less energy, produced less fecal matter, and expended less daily energy than uninfected grouse during timing associated with nematode maturation (Delahay & Speakman, 1995). In addition to the direct costs of nematode parasites upon nutrient availability and assimilation, parasites might also indirectly act as vectors for other detrimental parasites and pathogens. For example, sandflies of the subfamily *Phlebotominae* are the only vectors of diseases such as *Leishmaniasis* and transmit harmful *Plasmodium* to western fence lizards (Ready, 2013). Thus, it is worth noting that the possible negative effects of nematodes seen in these horned lizards might be in part due to nematodes acting as a pathogen vector, although no evidence for the worm as a vector currently exists.

As the effects of nematode load must also depend on the lizard's other energy demands, we must consider how reproductive energy costs will affect horned lizard ability to mitigate nematode infestation. Most lizards, including *Phrynosoma*, do not reabsorb the energy that they deposit into yolking follicles even if the female is stressed energetically (Goldberg, 2017); that is, they must endure high reproductive investment once they have begun vitellogenesis. Desert horned lizards also have massive clutch sizes compared to other lizards, devoting as much as 35% of their body weight in creating egg clutches (Pianka & Parker, 1975). Males also experience reproductive-associated energy costs such as testes enlargement and increased sperm production, though these costs are typically lower than in female lizards (Sherbrooke, 2003). In 12 years of radiotracking about 40 female horned lizards during their oviposition period in the Alvord Basin, a few females died just after egg laying and one died just before it would have oviposited; these deaths were anecdotally related to heavy nematode load (archived field notes 2004-2016, Anderson, 2019). Mortality could be related to the physiological stress of energy allocation when there is insufficient energy for both maintenance metabolism and reproduction. A study on seasonal variation in nematode size and abundance in the Alvord Basin shows that maturation of nematodes into gravid females occurred primarily in July and August and that juvenile nematodes were still present throughout the lizard activity season, indicating that the timing of this study (June-July) coincides with the seasonal peaks of both nematode infestation and reproductive energy costs (Hilsinger, Anderson, and Nayduch 2011).

Though *Skrjabinoptera phrynosoma* is the most common parasite of desert horned lizards, these lizards have also been found with other gastrointestinal, bloodborne and external parasites. A 2015 census of 104 desert horned lizards by Babero *et al.* (2015) shows that in addition to the four types of internal gastrointestinal nematodes and cestodes, lizards also contained five types of bloodborne protozoa and six types of external arthropods. There are multiple ways in which a primary coevolved parasite could influence the abundance of other parasites and pathogens infecting their host. For example, a coevolved parasite may decrease abundance of other parasites by providing interference competition. In cormorants infected with three helminth species there was demonstration of density-dependent effects both within species, where worm abundance was negatively correlated with size, and between-species, where abundance of the most common helminth species was negatively correlated with abundance of secondary and tertiary helminths (Dezfuli *et al.*, 2002). Presence of a chronic coevolved parasite could also increase abundance of other parasites by negatively affecting host immunocompetence. Dogs infected with immunosuppressive hookworms were more likely to present with other helminth parasites than uninfected dogs, and immunocompromised chickens were much less able to resist intestinal *Salmonella enteritidis* infestation than their immunocompetent peers (Loukas & Prociv, 2001, Carol & Grove, 1986, Arnold & Holt, 1995). Thus, it is worthwhile to examine all parasites within horned lizards, to gain a full understanding of their energetic challenges and to ascertain if nematode infestation can influence the greater parasitic community within their hosts.

Although it is valuable to examine ecological metrics such as home range size, feeding rate, and prey choice, it is also worthwhile to examine physiological metrics of health such as energetics and blood health to form a comprehensive assessment of lizard fitness. The ratio of packed cells to total blood volume (hematocrit) can be decreased by ectoparasites such as ticks or mites, though studies on the effect of helminth parasites on blood factors are limited (Dunlap & Mathies, 1993). Blood immune response can be elevated in cases of bloodborne infestation (Stacy, Alleman & Sayler, 2011), but can be reduced due to the immunomodulatory effects of some endoparasites (Maizels & Philipp, 1982).

Choice of metrics

In designing this study, I chose a variety of presumed fitness correlates of desert horned lizards: energetic ability for predator evasion, rate of prey capture and movement correlates for foraging and home range occupancy, and body condition in non-reproductive and reproductive lizards, and presumed immunity to infestation. It is expected that if nematodes elicit disadvantageous changes in fitness within their hosts, it will be demonstrated in these chosen metrics. Examining distance moved during foraging is as essential to assessing foraging success as are the direct measures of *number of ants* consumed and fecal size and mass, because locomotion-associated energy costs represents a considerable part of a lizard's energy budget (Anderson & Karasov 1981, Christian, Baudinette & Pamula, 1997). Examining sprint velocity and endurance should be a strong correlate of survival of free-living desert horned lizards because escape theory—the idea that prey will only flee when a predator becomes sufficiently close to maximize post-interaction fitness—holds true for even cryptic species, such as horned lizards (Cooper & Sherbrooke 2010). For animals living in nutrient-limited environments, maintaining glycogen, a sufficient matrix of body water, and fat pads can be essential to thriving in periods of low food availability or high reproductive costs (Henen, 1997). Thus, examining body condition (mass per unit body length) against nematode infestation provides crucial insight into severity of nematode infestation. Finally, examining hematocrit as a measure of oxygen storage and delivery capacity and examining relative leukocyte counts as a correlate of blood immune response to stress or infestation provides another set of metrics to help examine how well lizards endure nematode infestation.

Lizard body size affects many of the aforementioned metrics, including lizard energetics and foraging. For example, larger lizards forage longer distances and must eat more prey (Karasov & Anderson, 1984), and have a lower cost of transport (White & Anderson, 1994). Body size also tends to correlate with larger home ranges (Gad & Garland, 2002), and faster sprint speed (Bonine & Garland, 1999). Thus, each chosen response variable will be examined with both nematode load and body size measured as snout-vent-length, the length of the lizard's body from its snout to its cloaca, hereafter referred to as SVL.

As with all organisms, horned lizards endure constant and intense energy demands that affect their ability to survive, thrive, and reproduce. Although the average parasite load per lizard may not threaten the lizard population, it is expected that individuals with relatively large parasite loads will be harmed. The average infestation load within the lizard population may be an evolutionary compromise that allows for the continuation of both species and the co-evolution of the host-parasite relationship. By examining horned lizards fitness across a certain continuous intensity of nematode parasite *load* rather than simply comparing infected/uninfected animals, this study can yield a fuller understanding of helminth parasite effects in situ under the context of the suite of ecological, environmental and energetic costs desert horned lizards must endure to survive.

Questions and hypotheses

Question 1: What is the effect of nematode load on food acquisition behaviors of desert horned lizards such as: 1) their foraging distance measured as distance moved during foraging, 2) feeding rate measured as number of ants consumed and fecal size, and 3) percentage of prey that are *Pogonomyrmex californicus*, 4) home range size, and 5) their running velocity and endurance?

Hypothesis 1: Considering the reduced detrimental effects of coevolved parasites but high energy costs imposed by helminth parasites, I hypothesized that lizards with a medium nematode load would exhibit higher foraging distance and feeding rate than lizards with a low or high nematode load because a medium parasite load was low enough not to physically or energetically prevent the lizard from foraging, but was high enough to elicit a compensatory effect from the lizard to maintain adequate energy intake while also feeding its parasites. I hypothesized that above a certain threshold, greater nematode load would shorten foraging distance and reduce feeding rate due to weakness or illness. I hypothesized that although POCA consumption would remain a principal dietary component, lizards would consume fewer POCA at higher nematode loads as a potential avoidance response. I hypothesized that nematode load would not affect lizard home range size because such a link to has not been reasonably established, and that greater nematode load will reduce energetic speed and endurance due to direct nutrient loss to the parasites.

Question 2: What is the effect of nematode load on horned lizard body condition index (BCI) measured as mass per unit length?

Hypothesis 2: Because the study period will coincide with high nematode and reproductive-associated energetic costs, and because parasites limit the lizard's pre-absorptive nutrient intake, I hypothesized that lizards with a greater nematode load would have a poorer BCI than less parasitized lizards.

Question 3: What is the relationship between the abundance of the coevolved nematodes and the abundance of all other parasites, including ectoparasites such as ticks and mites, endoparasites such as cestodes, and bloodborne parasites?

Hypothesis 3: I hypothesized that as nematode load increases, numbers of other parasite would decrease due to interference competition elicited by the relatively large abundance of this primary parasite. However, at a sufficiently high level of nematode infestation, I hypothesized that the abundance of other parasites would increase because a severely infected lizard may be too unwell to resist infestation by other parasites or pathogens.

Question 4: What is the effect of nematode load on horned lizard blood health, specifically hematocrit and blood immune response?

Hypothesis 4: I hypothesized that hematocrit would be largely unaffected by nematode load in all but the most severe cases of infestation, where lizards would be suffering nematode-mediated illness. I hypothesized that blood immune response would be reduced in lizards with a high nematode load due to the immunosuppressive effect of some chronic parasites.

Question 5*:* Do the effects of nematode load differ between male and female lizards?

Hypothesis 5: Given the assumption of greater costs of reproduction in females, I hypothesized that females would have a greater feeding rate and foraging intensity than males to meet their energy demands, and the detrimental effects of increased nematode load would be more severe in female lizards, especially in metrics of energetic performance.

METHODS

Research overview

In June and July 2018, I examined fitness correlates of desert horned lizards varying in nematode parasite load in the northern extreme of the Great Basin Desert scrub in the Alvord Basin, Harney County, southeastern Oregon. Summer in the Alvord was the ideal time and place to conduct this M.S thesis research because a high-density population of desert horned lizards exhibiting a full range of nematode loads were also experiencing their seasonal peak of reproductive and nematode-associated energy demands.

With generous help from Dr. Anderson and students from Western Washington University courses BIO 408 (Ecological Methods) and BIO 409 (Research in Reptile Ecology), we captured 20 females and 19 males, and collected a combination of energetic, morphometric, and behavioral data with the goal of comparing these against parasite load. We measured 1) foraging distance as meters moved during peak foraging hours measured via radio-telemetry and physical sightings, 2) feeding rate as measured by fecal size and the number the nematode-infective ant species (POCA) and other ant prey species, 3) home range size also attained via radio-telemetry, 4) exercise performance in running velocity and endurance assessed on a racetrack, and 5) body condition index measured as body mass per unit body length. The search for other parasites of horned lizards included visual inspection for ectoparasites, digital examination of blood smears for blood parasites and other blood parameters and counts of parasites flushed from the stomach and cloaca with isosmotic saline. The independent variable of nematode load was calculated using a literature-based estimate of the sum of the mass-specific resting metabolic rate of all nematodes within a lizard. All response variables were examined for differences between the sexes via ANOVA, and their relationship to increasing nematode load and increasing snout-vent length by Type II OLS Regression. Using ANCOVA, I reduced the influence of snout-vent length as a covariate on all response variables and examined how nematode load alone affected these lizard's ability to survive, thrive and reproduce in an ecologically and energetically harsh environment.

Study Site, Methods Outline, and Timeline

We conducted this study in the northern extreme of the Great Basin Desert, at 1295 m elevation in the north-to-south trending Alvord Basin, a pluvial lakebed 100 km long by 15 km wide in Harney County, Oregon (Reheis, Adams, Oviatt, Bacon, 2013). The 16-hectare study site in xeric shrublands was dominated by big basin sage slightly upslope on sandy substrate and by greasewood downslope along dunes and hardpan flats. See ***Figure 35*** in the Appendix for location of the study site. After having obtained relevant permits from ODFW (permit #115-18); WWU ACUC (protocol #18-003), field research occurred from approximately June 27th to July 20th, 2018. Students enrolled in two concurrent WWU summer field research courses (BIOL 408 and 409) were essential to the capture, processing, fecal pellet collection, and radiotracking of lizards. Students working in pairs and teams followed a field data procurement protocol for each lizard encounter, and scribed data in detail in field notebooks (see Appendix ***Figure 36*** for sample field data). Capture records were verified within the lizard capture-and-release logbook, and the body data logbook (See ***Figures 37, 38*** for sample capture log, body data log).

Lizard Capture and Processing

We searched for lizards on the central nine hectares of the 16-hectare field course plot primarily from 0800-1100 and 1800- 2000 hours, when thermal conditions were known to be conducive to lizard activity and encountering lizards was most likely. Upon approach lizards would retreat to the cover of horizontal, near-ground stems and branches in the depths of shrubs whereupon hand-capture was generally easily accomplished. The lizard toe-clip identification (unique combination of toe clips, discussed below), presumed sex, age-and-size class (juvenile, subadult, adult), precise location of sighting, and the lizards behavior at time of sighting were recorded when captured (***Figure 36)***. Each lizard was kept individually in a uniquely labeled cloth bag, which was used as the primary identifier for that lizard for future processing. Upon return to camp, the lizard-containing bags were transferred to portable coolers with perforations to allow for airflow which were kept on the ground in the center of the full shade of a 3x6m shade tarp mounted horizontally 2m above the ground. The capture date, time, bag number, species, sex, age, toe clip, capture location, and scribes' initials were recorded in the lizard capture log (***Figure 37***).

Any female horned lizard that appeared to have an enlarged posterior portion of the abdomen was palpated by Dr. Anderson to determine whether she contained enlarged, yolking follicles or soft early-stage oviductal eggs, and could be held for a couple days to obtain fecal pellets, or she contained late-stage shelled, oviductal eggs and she needed to be measured and released immediately to allow for imminent oviposition. All lizards not immediately released were held for about three days to clear their digestive tract and allow for gut-empty body mass, and to obtain fecal pellets for fecal analyses ***(Figure 1)***. Lizard bags were inspected daily for fecal pellets and nematodes that may have emerged with those fecal pellets. After enough defecations to clear the gut contents, females were palpated for the presence, size and number of yolking follicles or oviductal eggs, and all lizards were palpated to estimate the mass of nematodes in the stomach. Lizards were weighed in a closed-environment digital balance to the nearest 0.01g, and snout-vent-length (SVL) and tail length were measured by Dr. Anderson to the nearest one-half millimeter. During weighing and measuring, a lighted jewelers loupe was used to find and count the number of ticks, mites, or any other ectoparasites present on each lizard's skin. If a lizard did not already possess a toe-clip combination for permanent individual identification, three-to-four toes (only one toe per foot) feet were clipped beyond the proximal phalanges (about midway along the length of the toe) with sharp surgical scissors coated with betadine, consistent with methods approved by ACUC Permit # 18-003. The two longest toes on the lizard's hind feet were not clipped as a precaution to not interfere with the lizards running ability, although evidence of such effect is lacking. Lizards were also marked with temporary non-toxic paint in a unique color combination of three 5mm transverse stripes across the lizard's dorsum, which would be lost with skin shedding. The color combination, read anterior-to-posterior, permitted rapid visual identification of lizards without the necessity of recapture or even a close approach of a free-ranging lizard (***Figure 2)***.

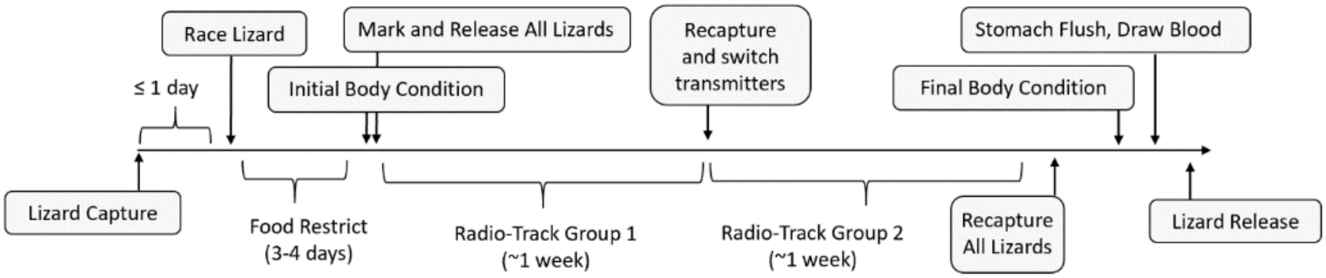


Figure 1: The overall timeline for all lizards being captured (with the bulk of capture happening before group 1 was radio-tracked) and the timeline for processing individual lizards who were not radio-tracked.

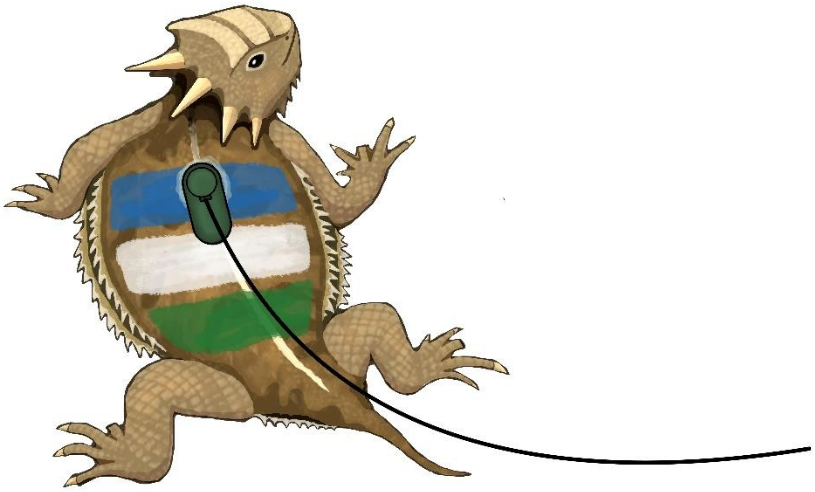


Figure 2: Visualization of horned lizard with permanent marking methods (toe clip), non-permanent marking methods (paint marks) and R1635 radio-telemetry backpack. A subset of 19 lizards (9 females and 10 males were radio-tracked.

Question 1: Food Acquisition Behaviors

Feeding Rate and Percent POCA

Daily rate of food intake was measured by two methods: 1) measuring average length and dry mass of daily-produced fecal pellets and 2) counting the number of prey based on examination of exoskeleton fragments of heads and other recognizable body parts (*e.g.*, legs and the elytra of beetles and insect legs) in fecal pellets. At least once per day, lizards were removed from their bags, and the bags were checked for fecal pellets. Intact fecal pellets were easily dropped into glass scintillation vial, non-intact pellets either were carefully removed either by flexible forceps (if fecal diameter could be determined) or carefully poured through a glass funnel into the scintillation vials. On occasion, if a lizard's fecal pellet was detected by palpation to be adjacent to or under the pelvic girdles, and the pellet seemed firm enough to be properly defecated, the pellet was gently expressed by placing the thumb transversely against the vent just anterior to the pellet, then rolling the thumb toward the pelvis-and-cloaca, thereby popping the pellet out the cloaca and directly into a scintillation vial. Expression provided a reliable determination of the presence of nematodes in the hindgut, as they could be pushed out along with the pellet. Upon return to WWU, fecal pellets were dried, weighed and measured, and all rocks, urates, and worms removed and weighed separately. The pellets or pellet fragments were placed in a glass petri dish and small forceps and probes were used to separate the arthropod fragments under a dissecting microscope. The heads of ants and other insects were separated from other parts, and all ant heads were identified to species and counted. At least three full-sized, relatively ant-filled fecal pellets per lizard were used to estimate daily feeding rate. To compare lizard prey choice with environmental ant availability, 21 pairs of pitfall traps (with each trap placed about 0.5m from the other in the pairing), which were half-filled with marine-RV antifreeze (principally ethanol and propylene glycol) were placed in open areas and collected after 7 days. Trapped insects were stored in ethanol and identified upon return to the lab. Pitfall traps were also placed under shrubs, but these pitfall traps were not included in this analysis because horned lizards most often forage in the open, and thus open pitfall traps will best reflect ants available to the horned lizards.

Foraging Distance and Home Range Size

Radiotracking

To assess the lizard's daily movement, foraging activity, and home range, 19 individuals (9 females and 10 males) were fitted with radio transmitters. Lizards were chosen based on their sex, size, estimated nematode load (determined by palpation of the prey-free stomach), and mesohabitat. The Advanced Telemetry Systems R1635 tracker (0.75grams, 6x14 mm) were affixed to the dorsum of the lizard using Ocyl-2 Cyanoacrylate consistent with ACUC Permit # 18-003 (Advanced Telemetry Systems, 2020). We positioned the radio-tracker just posterior to the thoracic girdle to not become caught on low-lying plant material and not interfere with the free movement of the head (***Figure 2***). Attempts were made to radio-track individuals four times per day: during mid-morning foraging activity (~ 0830-1030 hours), mid-day resting position (~ 1130-1630 hours), late-afternoon foraging activity (~ 1700- 2000 hours) and sleeping position (~ 2000 to 0700 hours). Sometimes the late-afternoon activity radio-tracking was skipped, and thus a minimum of two unique locations per daily activity period and an overnight location (end-day 1 = begin-day 2) were collected per each radio-tracked lizard each day. Every few mornings, each lizard was retrieved at its sleeping position before its begin-day basking, and the attachment condition of the radio transmitter was checked, glue re-applied as needed, and a fecal pellet was expressed and collected before the lizard had the chance to defecate early in the daily activity period. Optimally, lizards were tracked for about a week until at least 24 waypoints per lizard were created. Some lizards were tracked for fewer waypoints either due to the transmitter falling off and that lizard evading recapture, or due to the conclusion of the field course. About half the lizards were followed in the first week and the other half were followed in the next week.

Foraging distance

Upon return to the lab, all radio-telemetry waypoints were placed into consecutive order and recorded as “active” or “inactive based on time-of-day, temperature, and lizard behavior information recorded at the waypoint. Horned lizards demonstrate a predictable bimodal daily activity pattern, dictated by daily temperature, where they forage in the mornings and afternoons in temperatures conducive to foraging ability (Appendix ***Figure 39***). The distance between consecutive waypoints occurring within these blocks of activity was calculated from finding the absolute change in latitude and longitude between the points and calculating the hypotenuse of the resultant triangle using the Pythagorean theorem (***Figure 39***).

Home Range

Home ranges were computed by first visually inspecting a map of all waypoints using Google MyMaps and identifying the relatively few long-distance outliers as linear forays (e.g., single-episode, relatively linear-movement events by females on a one or two-day sojourn to lay eggs, or single-episode linear-movement events by males perhaps seeking a new home range) and removing those points from the data set, thus avoiding an erroneously large measure of home range size. This point removal was only relevant for two females who each had one foray during their radio-tracked period. The polygon-generating tool in MyMaps was then used to calculate the minimum convex polygon (MCP) home range in meters squared. By starting with a 3-waypoint polygon and measuring the MCP of that same lizards home range when each next consecutive waypoint is included, and seeing how many waypoints it takes before every next waypoint is included in the existing MCP (*i.e* when 100% of the MCP is reached with every new waypoint), I could predict the relative underestimation of the home range size (m^2) from its cumulative waypoints, ***Figure 3***.

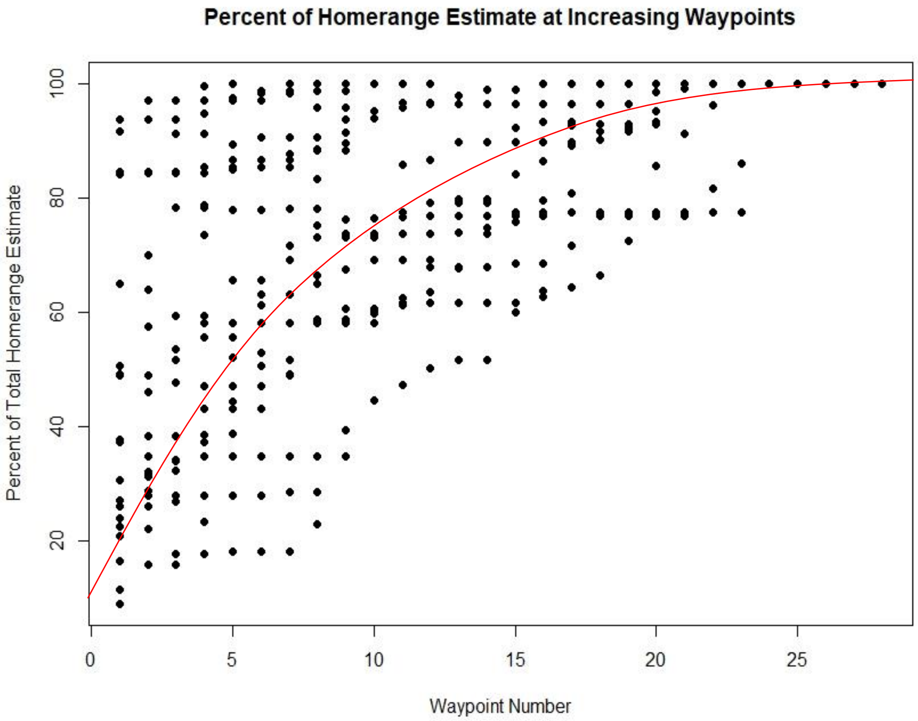


Figure 3: The asymptotic relationship of the percentage of the home range size (m^2) as increases with each additional consecutive waypoint.

Based on ***Figure 3,*** a minimum sample size of 15 waypoints in a home range seems needed to provide a reliable estimate of home range size, with a sample size of at least 20 being needed before the estimate is consistently approaching 100% home range. Most lizard home ranges were calculated with 15-25 waypoints, and a few were under 15 waypoints. For 52 home ranges available from multiple previous years of home range analysis, I calculated an estimated home range based on what that lizard's range would be if there were 24 waypoints been obtained, using the above asymptotic relationship. This technique was deemed an appropriate way to estimate home range since the estimated increase is relative to the existing home range for that individual. For example, a lizard with a home range of 100 m^2 from 15 waypoints (82% of total range based on ***Figure 3***) would be corrected using the following equation: $\frac{100\ square\ meters}{0.82} = 121$ m^2. Considering most home ranges were estimated from over 15 waypoints, most home ranges were only minimally increased by the estimation. This estimated home range is used as the metric in all home range analyses, since it allowed us to simulate a greater sample size rather than sacrificing samples with low waypoint numbers. These methods are consistent with the estimation of home range growth with increasing waypoints found in Christian and Waldschmidt, (1984).

Powder Tracking

While the high number of waypoints obtained during radio-tracking is helpful in determining lizard home range and average daily movement, powder-tracking captures fine movement detail which can be used as a more precise estimate of foraging-related moving effort. Among the 12 lizards chosen to be radio-tracked in 2018, we had the logistical ability to powder-track seven individuals. Strategic choice of which individuals to powder track was based on the goal of a balanced and varied mix of sexes, nematode load, and mesohabitat occupancy. Each lizard to be powder tracked was retrieved early in the morning, prior to its emergence and brought to base camp. Commercially available rabbit hide-and-fur—used for its fine hairs and low weight—was cut to shape to fit on the lizard's abdomen without restricting movement of moving limbs or the pelvic or pectoral girdles. A thin layer of Ocyl-2 Cyanoacrylate was used to apply the rabbit between the pectoral and pelvic girdles. Fluorescent, UV-reflective powder was applied heavily and worked into the rabbit fur, and then the lizard was released at its site of capture. On that day, the lizard was radio-tracked to its mid-morning foraging location and again to its heat-of-the-day resting location where powder was reapplied as necessary to maintain a distinguishable powder trail. When light levels were low enough for the UV reflection of the powder to be easily seen (about 45 minutes after sunset) we radio-tracked the lizard to its night-time sleeping position, where we then used UV lights to back-track the fluorescent trail left by the lizard to its morning release location. We used pin flags to mark every change in direction, with consecutive numbers or letters on flags denoting complex movements along the powder trail. To convert the powder trail into an interpretable form for later analysis, the powder trail was mapped on paper in real distances using a gridded measuring system. We used stakes and measuring tapes to grid the area of the powder trail into 5x5m squares. We measured the distance between positions and direction changes as indicated by consecutive pin flags, the distance from flags to nearby perennial plants, and the size and species ID of the plant or deadwood. We placed these locations onto the gridded map, ensuring accurate scaling with each grid length representing one meter. Using the times associated with the radio-tracked waypoints along the powder trail, the position of the sun and the approximate time the lizard was at each position was also noted so we could determine when lizards were sunlit or shaded. ***Figure 4*** demonstrates various points of the powder-tracking methods. Upon return to the lab, the digital scans of each powder track map were opened in AutoCAD, a grid unit was scaled to exactly one meter, and the powder track trail was measured in meters using the polyline tool. Though powder tracking sample size for 2018 is too small (n=7) for reliable analyses against SVL or nematode load, powder tracking can be used to assess the reliability of the higher sample size radio-tracking metrics. Powder tracking captures a higher movement resolution than radio-tracking and is assumed to be an accurate measure of distance moved during foraging. I chose 33 samples across 6 years for which both powder-tracking and radio-tracking data were available and compared those distances through a correlation test. Powder tracking distances averaged twice the distance of radio-tracking distances, and the two are reasonably well correlated (Pearson’s correlation coefficient =0.546, ***Figure 5****)*.

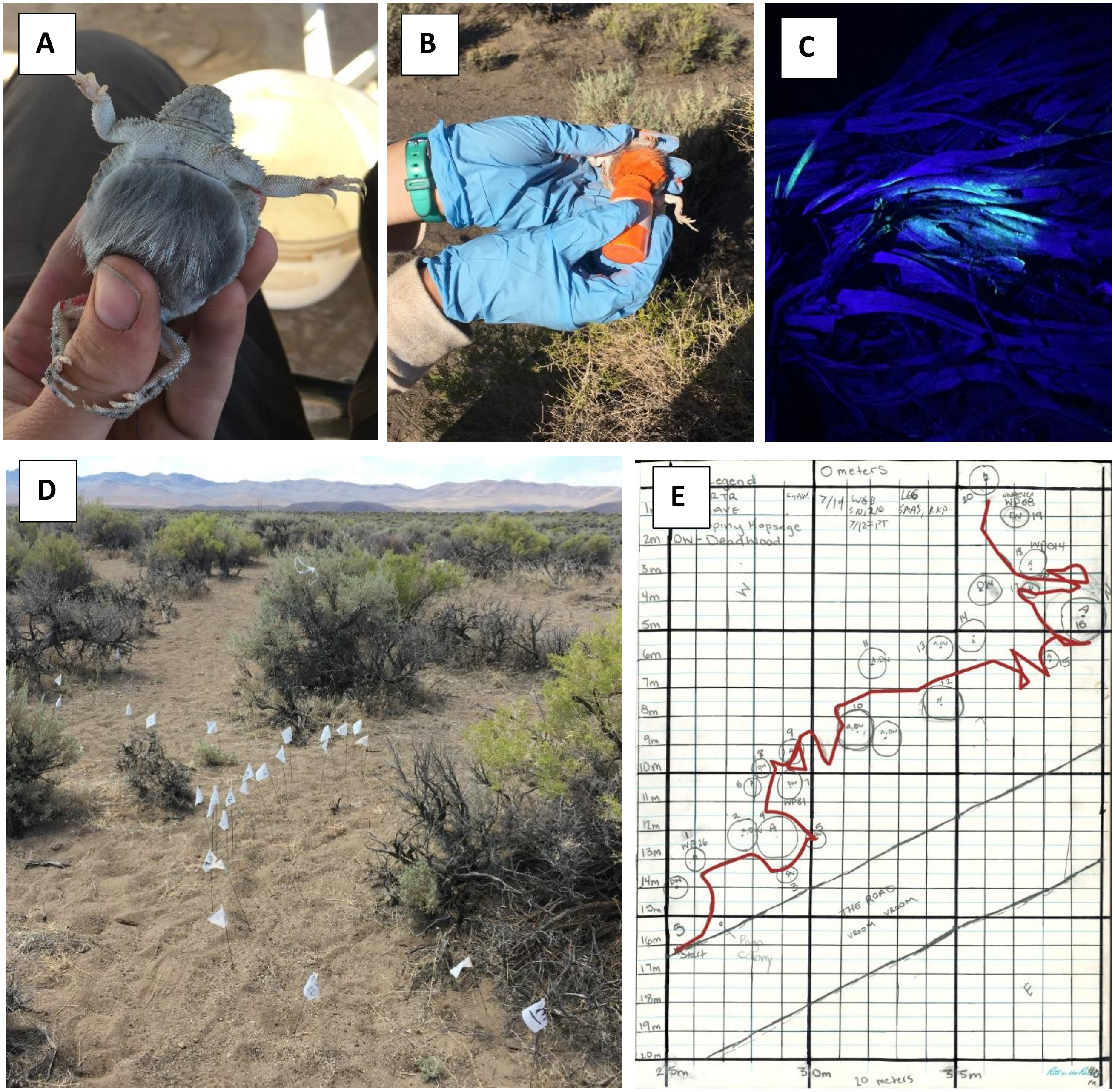


Figure 4: **A)** Rabbit fur cut to lizard size affixed using Ocyl-2 Cyanoacrylate. **B)** Rabbit fur covered thoroughly with fine-grained UV fluorescent powder. **C)** Fluorescent powder trail detected at night with UV flashlights and marked with pin flags **D)** Pin flag locations documented on paper the next day. **E)** Digital scan of powder map, each 1x1cm square represents a meter, lizard track digitally marked and measured in meters.

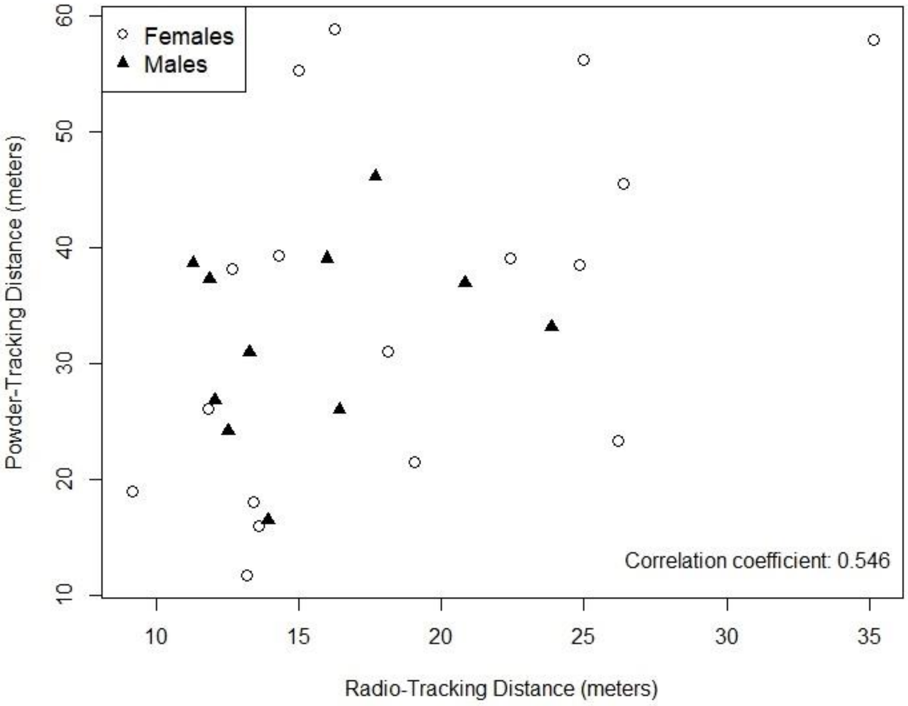


Figure 5: Correlation between powder-tracking distances and radio-tracking distances. n=33, 2007 n=9, 2009 n=5, 2011 n=4, 2012 n=6, 2015 n=3, 2018 n=7, Note the differences in scale, powder tracking distances are an average of double the radio-tracking distance.

Short-sprint speed and long-sprint endurance

Lizards held at field-active body temperatures between 35-40 °C, in thermostatic insulating chambers were raced within 24 hours of capture to ascertain their maximum velocity and running endurance. Racing was done in either mid-morning or mid-afternoon when the substrate of the racetrack was in full shade but air and ground temperatures were no lower than 32° C or higher than 40° C. Lizard body temperature was taken via cloacal thermometer directly before racing to ensure optimal field active body temps, averaging 38 ± 2 °C, consistent with Dr. Anderson's direction and studies on thermoregulation within *Phrynosomatidae* (Lara-Resendiz *et al.* 2014).

The raceway was 20m long, 0.6m wide, oriented north-to-south, made of 0.6 m tall aluminum flashing with the *in situ* sandy soil surface substratum raked and swept into a level, smooth, and relatively homogenous surface. For horned lizards, only the southern 12m was used. The central 10m of that section was used for video-recording, with a 1m section on each end serving as a slow-down buffer with a collection of small branches of common shrubs to serve as refugia, giving lizards a visually obvious target towards which to run. The lizard was released and chased by me, hand-clapping and shuffle-stomping behind the lizard to encourage a continuous run straight towards the refugium at the end of the track. Once the lizard crossed the 10-meter mark, it was quickly turned around and released at the same 10-meter mark to run the other direction. A stopwatch was started when the lizard was released and stopped every time the lizard was being turned around. Once the lizard was unwilling to run (determined for all lizards as remaining still despite at least 3 seconds of continued startling) the stopwatch was stopped, and the trial was concluded. Two grayscale high-speed GoPro cameras on tripods mounted over the racetrack recorded the lizard’s path while meter marks were in view, which was used to digitally measure maximum velocity in meters/second. A video camera on a tripod at the end of the racetrack recorded narrated data of the lizard’s bag number, the ground temperature of the racetrack, the body temperature of the lizard as well as start and stop times, and times where a lizard was turned after having run the 10-meter length of the track, and the final summed distance run before exhaustion.

Question 2: Body Condition

I chose to explore two indices of body condition. The first is a simple body condition index, composed of mass divided by SVL, the second is the scaled body mass index, calculated by:

Equation for scaled body condition

$$SMI = \left(Mass_I * \frac{SVL_P}{SVL_I}\right)^m$$

Where $\underline{Mass_I}$ is the mass of the individual, $\underline{SVL_P}$ is the SVL of the population (all *P. platyrhinos* measured for SVL), and $\underline{SVL_I}$ is the SVL of the individual. This term is all raised to $\underline{m}$, which is the slope of the regression of natural log of the population's mass to natural log of the population's SVL. Lizards were held for 3-4 days after capture to allow for the gastrointestinal tract to be cleared of food and thus, only post-absorptive body masses were used. Simple body condition index is preferable for its simplicity and intuitive interpretation, while the scaled index can be beneficial in that it is a ranking of individual lizards within their population (Peig and Green, 2010, Bartlett *et al.*, 2015). Both metrics were to be explored, and as were no differences in trends between the two, the simple mass index was used.

Question 3: Parasites other than *S. phrynosoma*

Endoparasites

After the lizards were allowed to exist in their environment for about a week to be radio-tracked, they were recaptured and gastrointestinal endoparasites such as full nematodes and cestodes proglottids were removed via gastric and cloacal lavage, respectively**.** First, the lizard was weighed to get their pre-flush mass. Then, a solution of isosmotic saline and other electrolytes (unflavored Pedialyte TM) was drawn into a 10 ml syringe, which was attached to a 5 cm long section of 2 mm wide silicon food grade tubing. This tube was gently inserted into the mouth and fed down the esophagus, stopping when a palpating finger detected the tube at the base of the pyloric stomach. The Pedialyte TM was then slowly administered through the syringe as the stomach was palpated posteriorly to anteriorly to gently work the contents up through the digestive tract until it was expressed through the mouth. Typically, two-to-three 10ml rounds of flushing were sufficient to clear the stomach of all contents. The stomach contents were caught in a funnel and collected into a glass scintillation vial and stored in 95% ethanol, consistent with Justine, Briand and Bray, (2012). Cloacal flushing was performed similarly, with a separate syringe and tube being inserted into the cloaca, and several rounds of isosmotic saline being administered and released until the cloaca was determined to be clear. Cloacal contents were stored with the stomach contents since the parasites of interest (cestode proglottids and nematodes, respectively) can be easily identified later in the laboratory.

Nematode Load

The raw nematode count was converted into a quantitative measure of energetic expense by calculating the summed basal metabolic rate of all nematodes within a lizard. First, nematodes were separated from the rest of the stomach contents and sorted based on obvious size groupings. The length and largest diameter in mm of two or three vouchers from each group were measured under a dissection microscope and averaged into representative group metrics, and the number of nematodes of each size group was recorded. The mass of each nematodes within each group was calculated using the Andrassy formula for massing nematodes.

Andrassy formula for the mass of nematodes

$$\boldsymbol{m} = \frac{(\mathrm{l} * d^2)}{1.6 * 10^4}$$

Where ***m*** is fresh mass in grams, *l* is the is nematode length in mm, *d* is the largest diameter across the nematode in mm, and $1.6\mathrm{x}10^4$ is a constant associated with the density of nematodes and the volume of a double tapered cylinder (Andrassy, 1956). This value of mass was then used to calculate the resting metabolic rate in joules/hour based on metabolic equations for parasitic helminths (Hechinger, 2012).

Question 4: Blood Features

Just prior to final lizard release, a 10-30 microliter blood sample was collected from the post-ocular sinus by sliding a heparinized capillary tube laterally and posteriorly into the posterior corner of the eye socket, thus penetrating the orbital sinus, a technique commonly used for rodents, birds and lizards (BVA/FRAME/RSPCA/UFAW, 1993; Van Herk *et al.*, 1998). The blood was transferred to a 20 microliter Eppendorf tube, a blood smear was made on a clean glass slide, air-dried, and placed in a safe storage container, consistent with Linne and Ringstud, (1999). The blood not used for the slide remained in the 20 microliter Eppendorf tube and stored under ice until my return to the lab.

Hematocrit

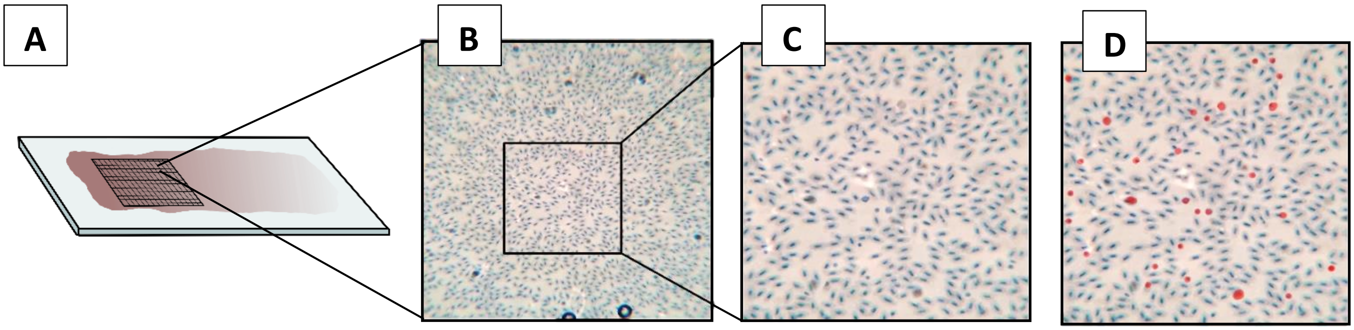
Upon my return to WWU, hematocrit (percentage of blood occupied by solid cells) was determined by gently mixing the still-liquid blood within the Eppendorf tube using a laboratory vortex, loading a heparinized capillary tube 2/3 full with the mixed blood, capping the end with waterproof polymer clay, and centrifuging for 5 minutes at 10,000 rpm. The length of the pellet of separated packed cells was measured with digital dial calipers and divided by the length of all the blood components to achieve the ratio of packed cells to total cell volume, consistent with (Bull, *et al*, 2000).

Bloodborne Parasites and Blood Immune Response.

Air-dried blood slides were stained using standard protocol with Wright-Giemsa stain, and a clear square grid sticker with 20 1mm sections per side was placed on an evenly strained, medium-density section of the slide. Using a compound microscope ocular camera attachment, a picture was taken of 10 haphazardly chosen, seemingly representative and visually average 1x1mm sections at 40x. These images were then opened in Adobe Photoshop and the number of cells on each side was counted and multiplied to obtain the approximate number of cells per 1mm square. Every visible immune component (leukocyte) was digitally marked, making sure not to count clotting agents such as collections of thrombocytes.

Immune components were identified using figures from a study of hematology of Texas horned lizards (*Phrynosoma cornutum*) (McEntire *et al.*, 2018). The total number of leukocytes was counted, subtracted from the total cell count to get the number of erythrocytes, and the number of leucocytes per 1000 erythrocytes was used as the final metric for analyses. I also attempted to identify any blood-borne parasites and pathogens using a guide to the diagnostic hematology of reptiles (Stacy, Alleman and Sayler, 2011).

Lizard with Average (~50) Leukocytes per 1000 Erythrocytes



Outlier Lizard with highly elevated (~150) Leukocytes per 1000

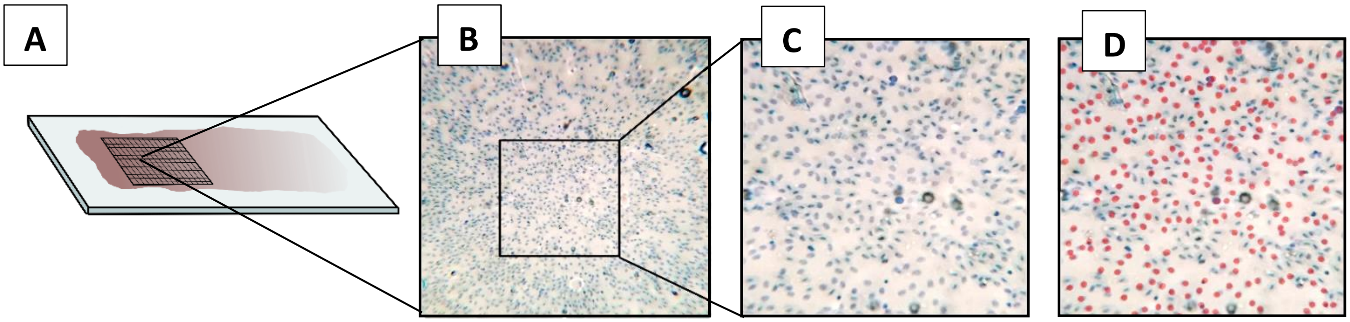


Figure 6: Methods for counting blood immune response. **A)** 100x100mm^2 grid sticker was applied to a section of slide with an evenly distributed array of cells. **B)** One of ten random 1mm^2 sections in that array that were photographed under 40x. **C)** The photograph of that section magnified digitally to count leukocytes (note how leukocytes have a more variable size and lack the distinct shape and bi-coloration of erythrocytes). **D)** Leukocytes were digitally highlighted and counted for the entire 1x1mm section, leukocytes per 1000 erythrocytes were averaged for ten 1mm^2 sections.

Statistical Methods

Using the statistical program R, data sets were first examined to meet the assumptions of parametric testing for normality and homogeneity of variance between groups using the Shapiro-Wilkes normality test and Levene's test, respectively. If non-normal, a single natural-log transformation of the data usually sufficed to achieve normality. After transformation, a one-way ANOVA was performed to see if the response variable significantly varied across the primary categorical independent variable-sex. For all statistical analyses α was set at 0.05. If the difference was statistically significant, those data were separated into male and female subsets to examine the effect of nematode load and SVL for each sex. If there was no significant difference between sexes, data were pooled for further analysis (***Figure 7***).

Because each variable is associated with error (*e.g*., nematode load, snout-vent length) and response variable (*e.g*., maximum running velocity, endurance, body condition) expected to correlate to some degree with nematode load and SVL, a Type II Ordinary Least Squares regression was used to examine the relationship between nematode load, SVL, and each response variable. Analysis of Covariance (ANCOVA) was used to correct for the influence of SVL as a covariate. As ANCOVA requires a categorical independent variable to compare groups, nematode load was examined via histogram to ascertain if there were any intuitive groupings that could be formed (see ***Figure 8****)*.

Nematode load demonstrates a negative binomial distribution characteristic of parasite abundance (Rabajante, Anzia & Gokhale, 2020) (***Figure 8 (A)***). While this is worthwhile to note for its implications to parasite-host ecology, transforming nematode load is necessary to meet the assumption of normality for parametric statistical analyses. Based on ***Figure 8 (B)*** I formed three groupings of nematode load, (0-2.2 joules/hour, 2.2-3.2 joules/hour, and 3.3+ joules/hour, low n=12, medium n=13, and high n=15). In analyzing nematode load against each response variable, I used R to determine if the covariate was significant and adjusted the mean for each category to eliminate the effect of SVL. Those SVL-corrected means were plotted as a bar graph with 95% confidence intervals expressed as error bars.

Statistical Methods Flowchart

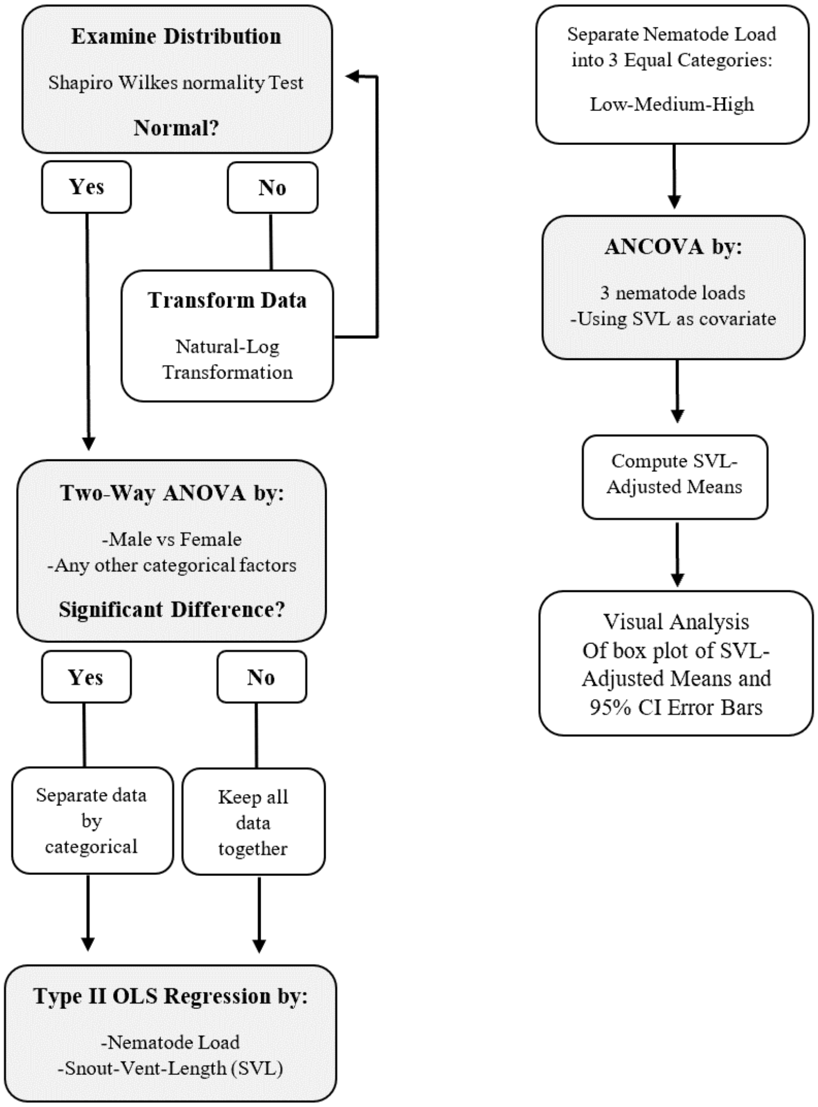


Figure 7: Flowchart of the path of analyses of any single response variable, one path starting with examining distributions to satisfy the assumptions of parametric testing and ending with running Type II OLS Regressions by both Nematode load and SVL. The other path starts with a categorized metric of nematode load and examines each response variable by those categories using SVL as a covariate via ANCOVA.

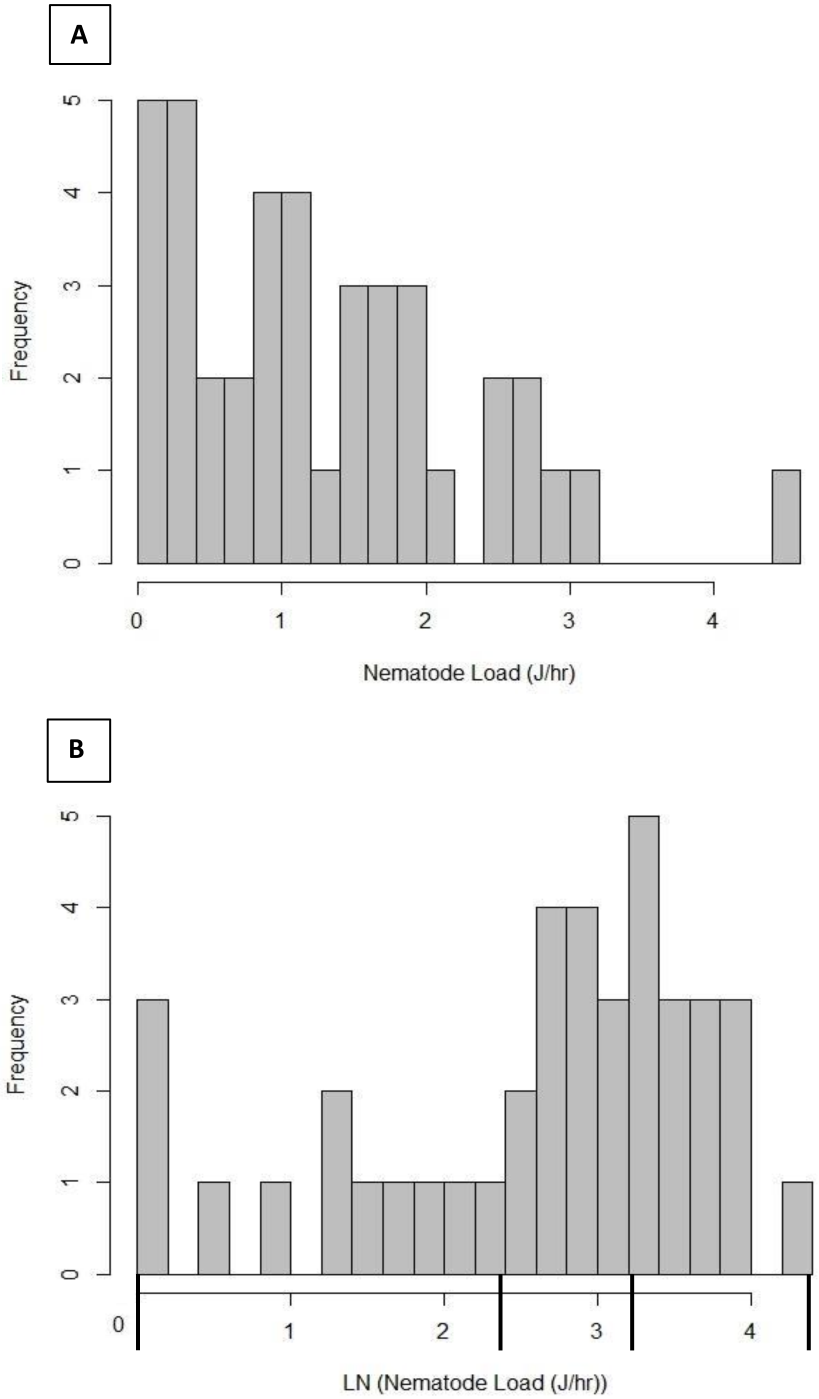


Figure 8: Histogram of: **A)** distribution of raw nematode load demonstrating a negative binomial distribution and **B)** distribution of the log-transformed nematode demonstrating an approximately normal distribution. Signal lines on the x-axis indicate the breakdown of nematode load into 3 levels for the purposes of analysis of covariance (ANCOVA).

RESULTS

General patterns of nematode load and morphometrics

Female lizards averaged 15mm longer in SVL than males among the 191 individuals examined for body size between 2015-2018 (ANOVA, df=(1,189), F= 60.71, p>0.001, η^2=0.243, females n=98, males n=93, ***Figure 9****)*. Females and males were not significantly different in nematode load, which was only taken in 2018 (ANOVA, df=(1,38), F=0.096, p=0.34, η^2=0.023, females n=20, males n=19, ***Figure 10****)*. There was not a significant positive relationship between SVL and nematode load in female lizards (Type 2 OLS, p=0.25, r^2= 0.026, n=20) though there was a significant positive relationship in male lizards (Type 2 OLS, p=0.013 r^2= 0.273, n=19, ***Figure 11****)*. For all analyses, nematode load refers to the log-transformed nematode load.

Question 1: Food Acquisition Behaviors

Foraging Distance

Males and females tend moved about the same distance during daily foraging activity (ANOVA, df=(1,17), F= 0.082, p=0.778, η^2=0.004, females n=9, males n=10). There was a significant positive relationship between foraging distance and nematode load, (Type 2 OLS, p=0.017, r^2= 0.238, n=19), as well as between foraging distance and SVL (Type 2 OLS, p=0.031, r^2= 0.191). When correcting for SVL as a covariate, lizards with a greater nematode load had a longer foraging distance than those with a smaller nematode load (ANCOVA, df=(2,1,15), load p=0.057, svl p=0.632, n=19, ***Figure 12****)*.

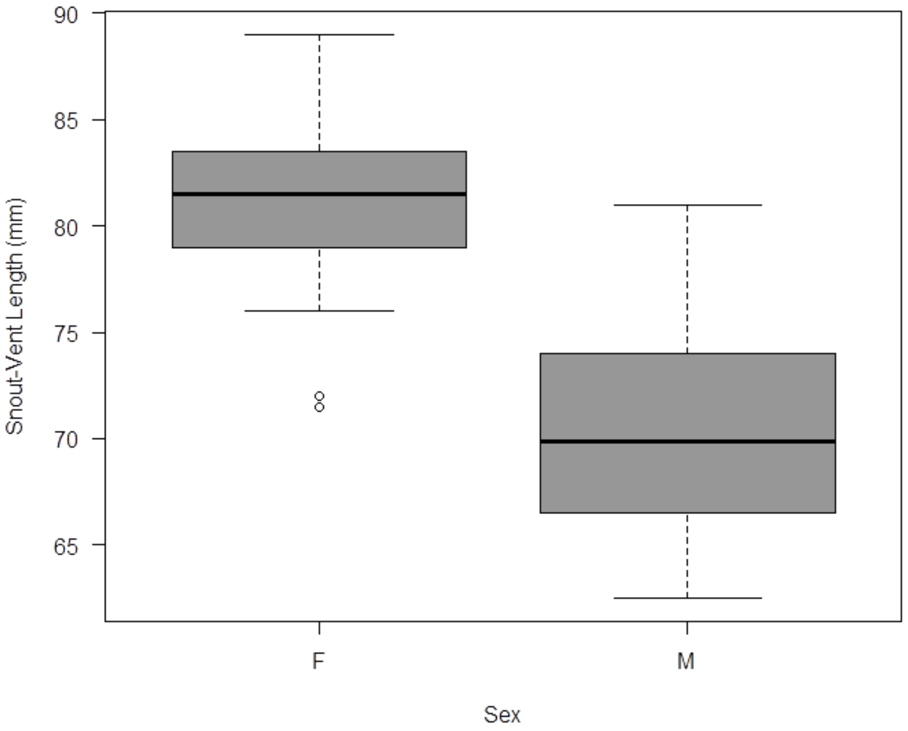


Figure 9: Snout-vent length (SVL) of female vs male lizards.

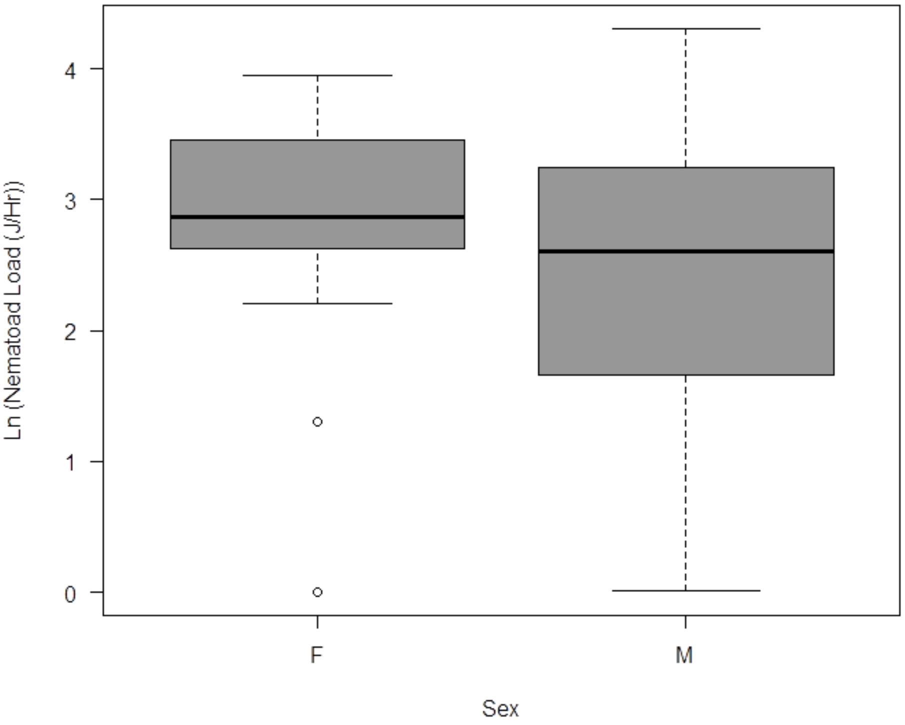


Figure 10: Log-transformed nematode load energetic expense for female vs male lizards.

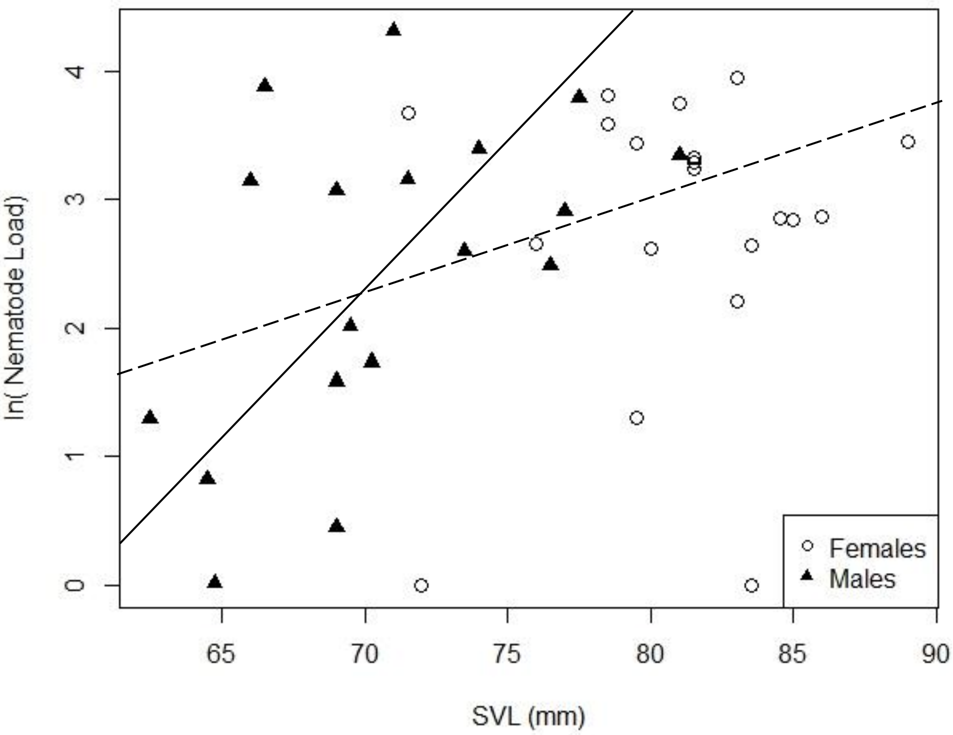


Figure 11: Type II Ordinary Least Squares (OLS) Regression of SVL by ln (nematode load) in for horned lizards. Dashed line is trend line for female lizards, solid line is trend line for male lizards.

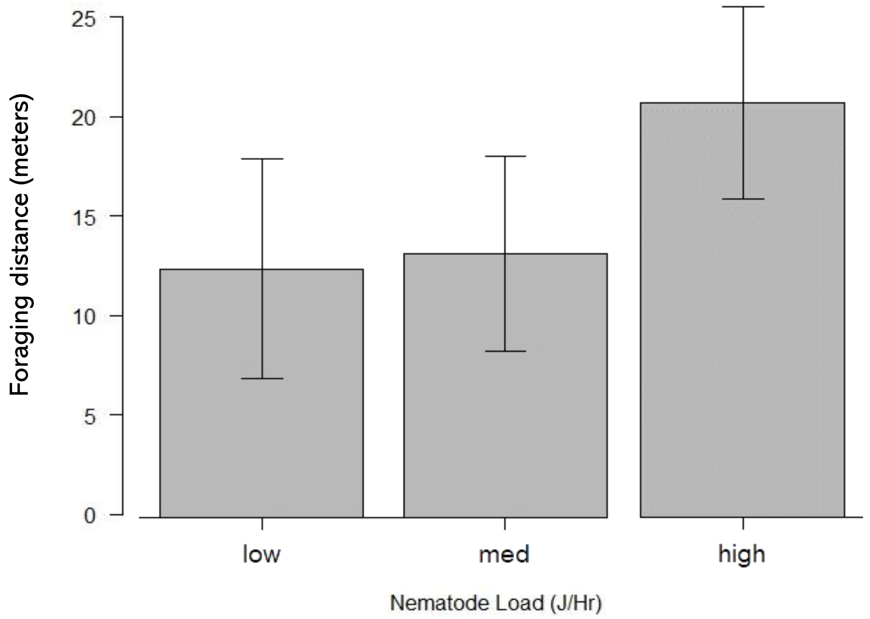


Figure 12: Foraging distance in meters (adjusted for body size) across nematode load categories from low to high nematode load. Error bars represent 95% confidence intervals.

Feeding Rate

Total number of ants per fecal pellet was not different between the sexes (ANOVA, df=(1,24), F=1.035, p=0.319, η^2=0.145, females n=12, males n=14) and was not significantly associated with either nematode load (Type 2 OLS, p=0.120, r^2= 0.080, n=26) or snout-vent length (Type 2 OLS p=0.272, r^2= 0.016, n=26). Although number of ants consumed did not differ between the sexes, females had significantly longer and heavier fecal pellets than males (ANOVA, length: df=(1,26), F= 5.74, p=0.024, η^2=0.146, mass: df=(1,26), F=4.35, p=0.048, η^2=0.159, females n=16, males n=12, ***Figure 13***). Fecal length and mass both significantly increased with SVL (Type 2 OLS, length: p=0.007, r^2=0.236, n=28, mass: p=0.008, r^2=0.215, n=28), but neither had a significant relationship with nematode load, (Type 2 OLS, length: p=0.110, r^2=0.057, n=28, mass: p=0.071, r^2=0.086, n=28). When corrected for the influence of SVL as a covariate via ANCOVA, males showed no difference in fecal mass or length among low, medium and high nematode loads. Females, however, produced heavier and longer fecal pellets at low nematode loads but produced lighter and shorter fecal pellets at medium and high nematode loads, though variance in mass is too high to be definitive (ANCOVA, length: df=(2,1,12), load p=0.136, svl p=0.002, n=16, mass: df=(2,1,9), load p=0.431, svl p=0.364, n=12, ***Figure 14***).

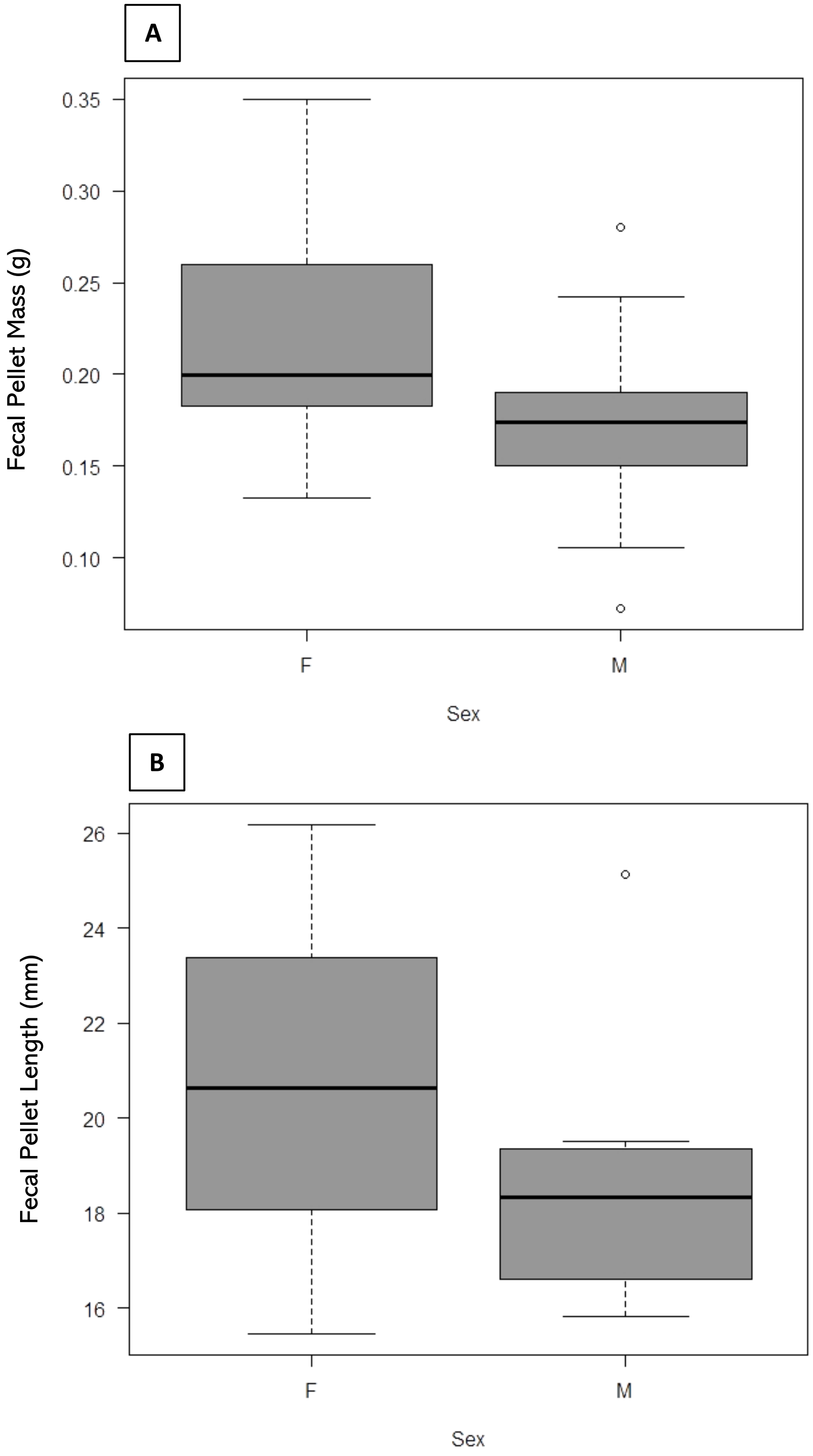


Figure 13: A) fecal mass in grams B) fecal length in mm of female and male horned lizards.

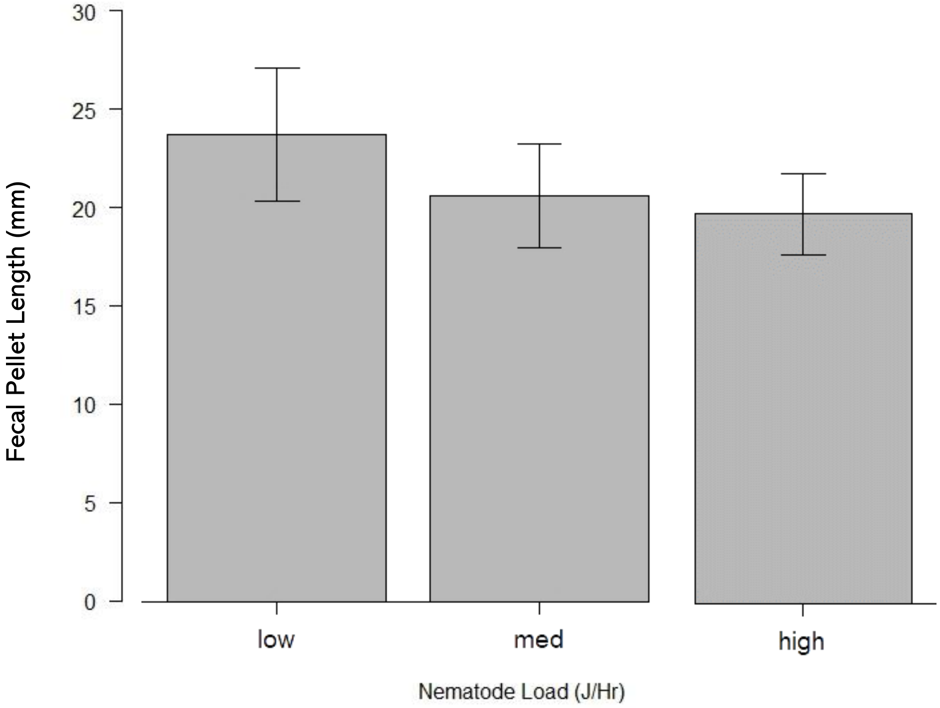


Figure 14: Fecal length (adjusted for body size) in female lizards across nematode load categories low from low to high nematode load. Error bars represent 95% confidence intervals.

Prey Choice (Percent POCA)

Concerning prey choice, females consumed significantly more POCA, almost twice as much as males (ANOVA, df=(1,123), F=36.965, p=>0.001, η^2=0.231, females n=61, males n=63, data from 2015-2018, ***Figure 15***). Female lizards with longer snout-vent-lengths consumed significantly more POCA (Type 2 OLS, p=>0.001, r^2= 0.171, n=61, ***Figure 16***). In female lizards, POCA consumption increased significantly with increasing reproductive investment, appearing higher in lizards with early or late yolking follicles than in lizards who are non-reproductive or post-reproductive, (ANOVA, df=(4,80), F=20.42, p=>0.001, η^2=0.505, Male n=37, NR=Non-reproductive, n=45, EYF= early yolking follicles, n=16, LYF=Late yolking follicles, n=18, PR=Post-Reproductive, n=19. ***Figure 17*****).**

In contrast to female lizards, POCA consumption in males did not change with snout-vent-length, (Type 2 OLS, p=0.430, r^2= 0.0004, n=63, ***Figure 16***). Increased POCA consumption was not associated with an increase in the total number of nematodes within a lizard (Type 2 OLS, p=0.224, r^2=0.029, n=19). In both sexes, when corrected for the influence of SVL, lizards with high and medium nematode load consumed fewer POCA than lizards with a low nematode load, more dramatically so in females, (ANCOVA females: df=(2,1,8), load p=0.025, svl p=0.113, n=12, males: df=(2,1,9), load p=0.452, svl p=0.866, n=14, ***Figure 18***). When comparing distributions among species of prey in pellets in 2018 fecal pellets (n=26) to the relative distribution of ant species assessed via open pitfall traps (n=42), desert horned lizards preferred the four common ant species (*Pogonomyrmex californicus, Myrmecocystus kennedyi, Crematogaster mormonum,* and *Camponotus hyatti*) which compose 66% of available ant species but 90% of the lizard's diet. Furthermore, the only major difference between the diet of males and females was that for primary prey, males prefer *Myrmecocystus kennedyi,* while females prefer *Pogonomyrmex californicus* (***Figure 19***).

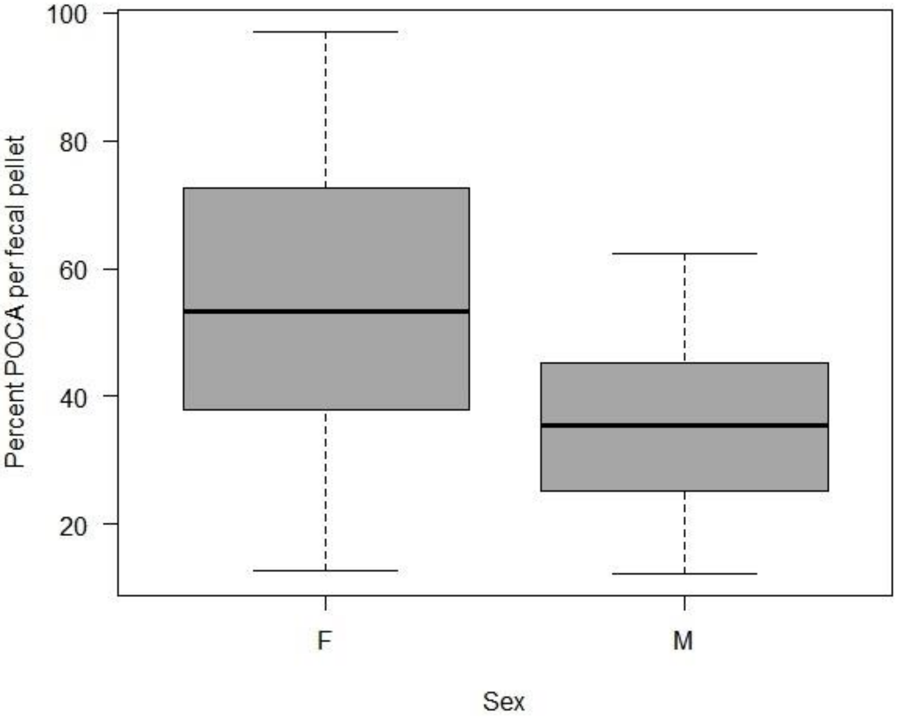


Figure 15: Percentage of the fecal pellet represented by *Pogonomyrmex californicus* (POCA) in the average fecal pellet between females and males, data from 2015-2018.

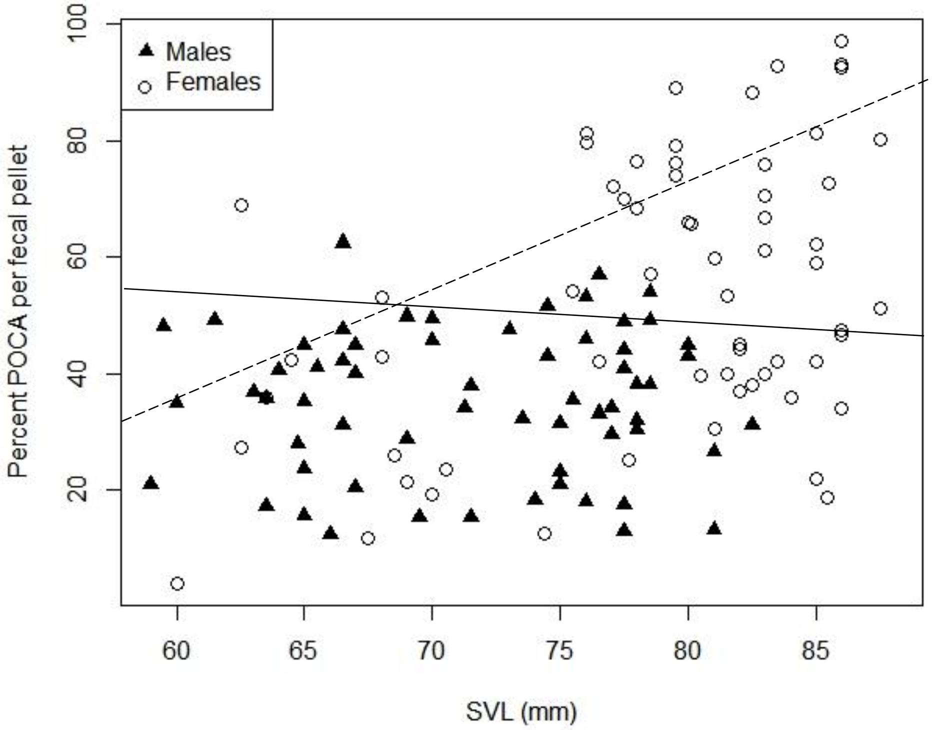


Figure 16: Type II OLS Regression of percent of POCA out of all ants per fecal pellet by SVL for male and female horned lizards. Dashed line is trend line for female lizards, solid line is trend line for male lizards. Data from 2015-2018.

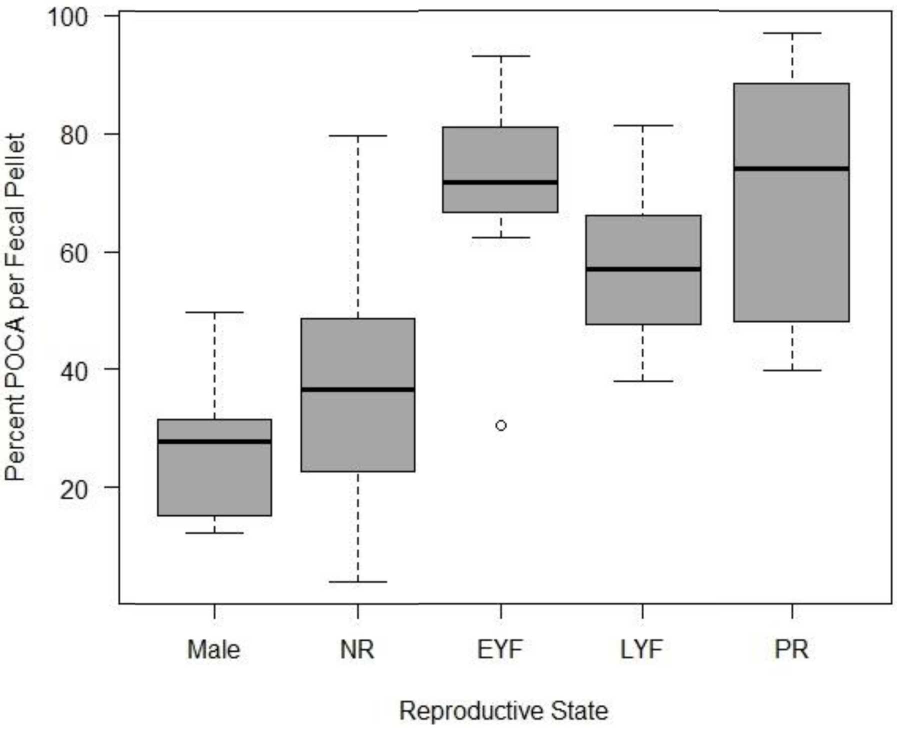


Figure 17: Percentage of POCA out of ant species in fecal pellets of males and females across reproductive states from lizards captured 2015-2018. NR=Non-reproductive, EYF= early yolking follicles, LYF=Late yolking follicles, PR=Post-Reproductive.

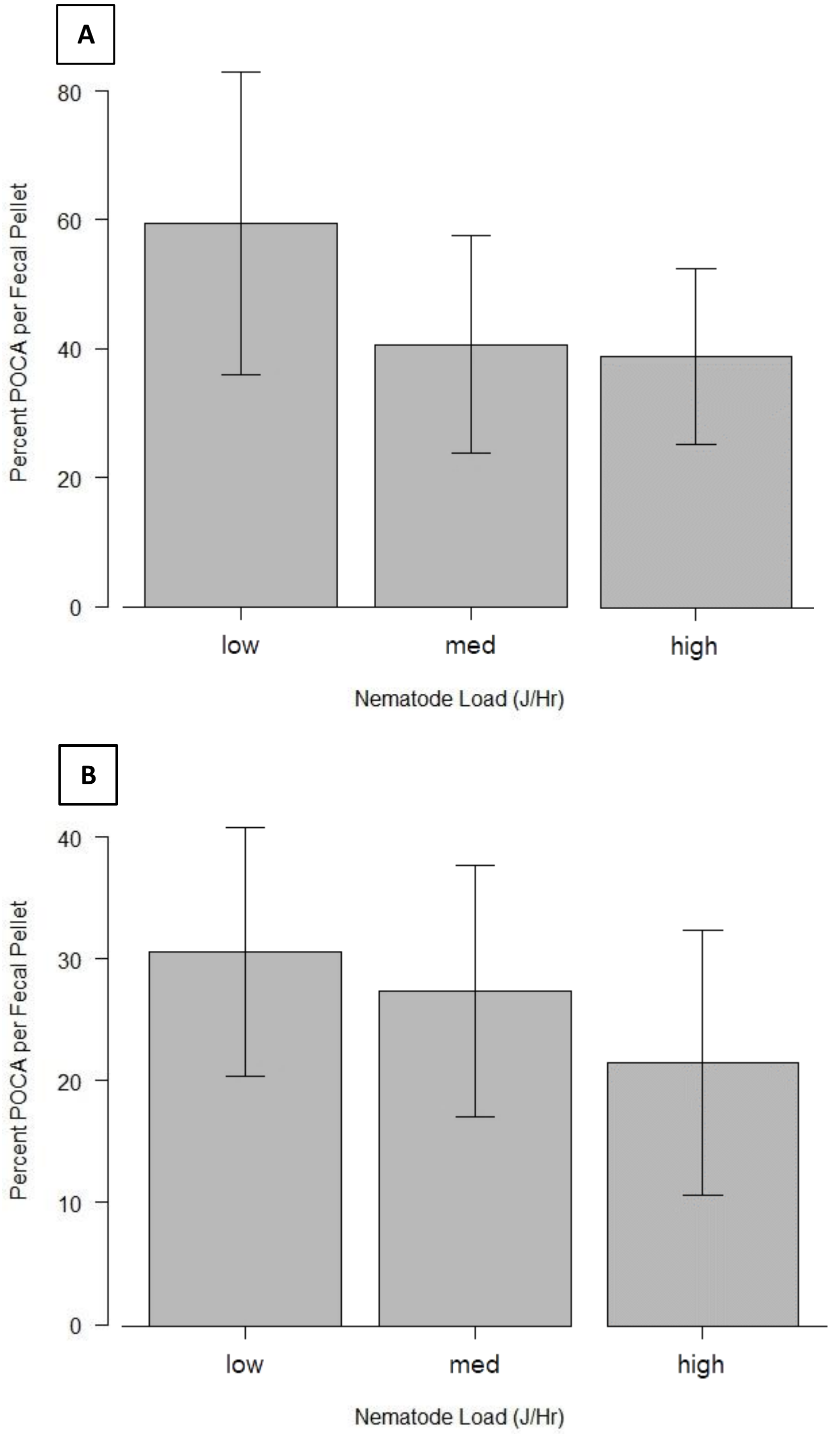


Figure 18: Percentage of POCA out of ant species in fecal pellets (adjusted for body size) for A) female and B) male horned lizards across nematode load categories, error bars represent 95% confidence intervals.

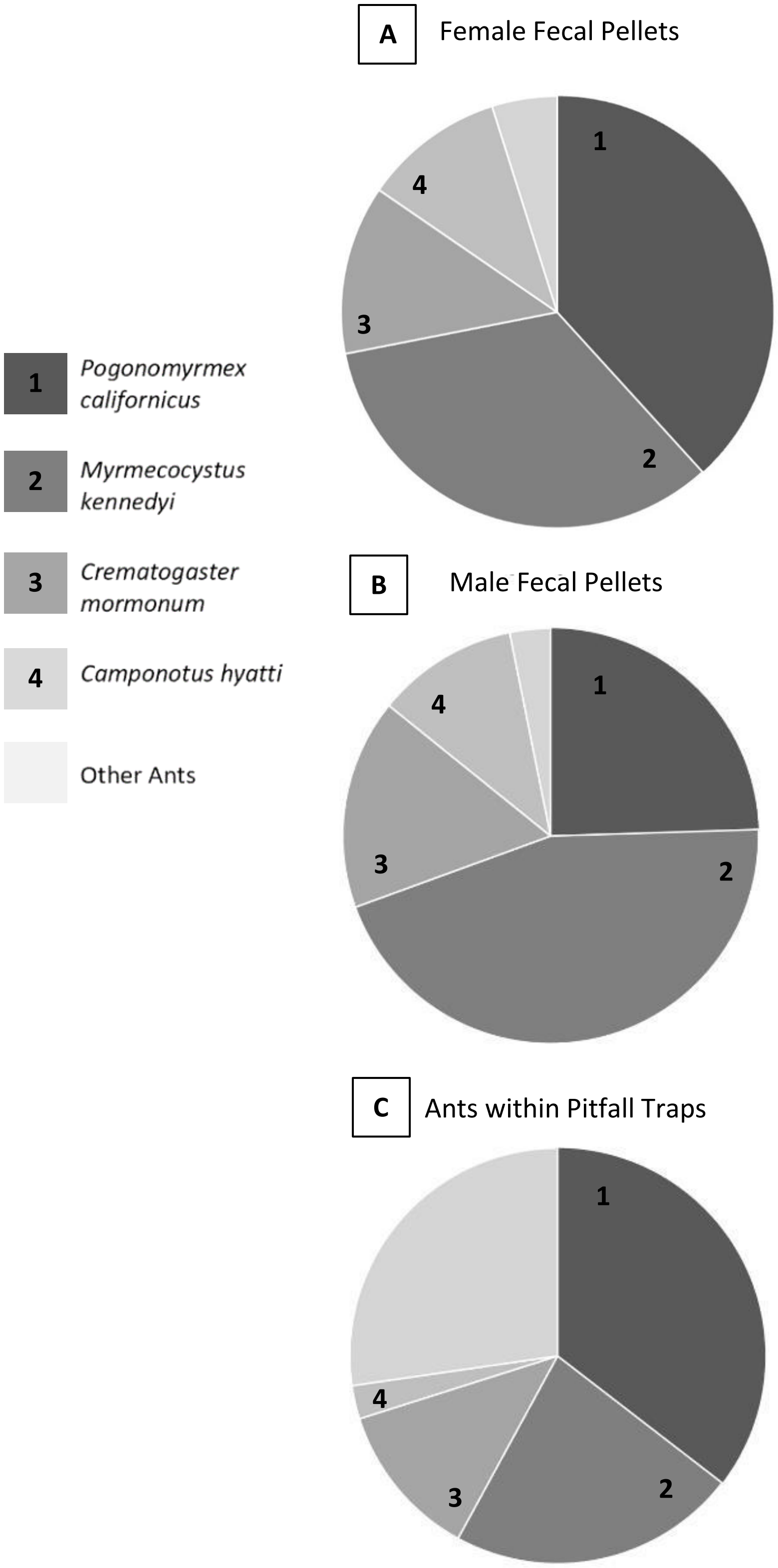


Figure 19: Proportion of the four most common ant species in **A)** fecal pellets of female lizards. **B)** fecal pellets of male horned lizards, and **C)** the proportion of those same ants in the environment assessed from pitfall traps. A more detailed breakdown of all species can be found in the appendix ***Figure 40*.**

Home Range Size

Home ranges of females might have been larger than male home ranges (ANOVA, df=(1,50), F=3.05 p=0.086 η^2=0.057, females n=26, males n=26, ***Figure 20***). Data are from 5 years spanning 2007-2018. Home range sizes had a marginally insignificant positive relationship with nematode load (Type 2 OLS, p=0.052, r^2= 0.156, n=18) and a significant positive relationship with SVL (Type 2 OLS, p=0.028, r^2= 0.207, n=51). Corrected for SVL as a covariate via ANCOVA, lizards with the highest nematodes may have had a larger mean home range, but variance is too high to claim significance (ANCOVA, df=(2,1,12), load p=0.2097, svl p=0.599, n=18, ***Figure 21***). A satellite view of the field site superimposed with lizard home ranges and radio-tracking waypoints can be found in ***Figure 22.***

Endurance and Velocity

Maximum velocity attained on the racetrack was not different between males and females (ANOVA, df=(1,20), F=0.128, p=0.723, η^2=0.0098, females n=12, males n=10) and showed no association with nematode load (Type 2 OLS, p=0.435, r^2=0.001), or SVL (Type 2 OLS, p=0.166, r^2= 0.04). Running endurance also did not differ between the sexes (ANOVA, df=(1,20), F=0.199, p=0.66, η^2=0.0098, females n=12, males n=10). Endurance strongly increased with snout-vent-length (Type 2 OLS, p=0.026, r^2=0.17). When corrected for the effect of SVL as a covariate, lizards running endurance significantly decreases with increasing nematode load categories (ANCOVA, df=(2,1,18), load p=0.015, svl p=0.032, ***Figure 23***).

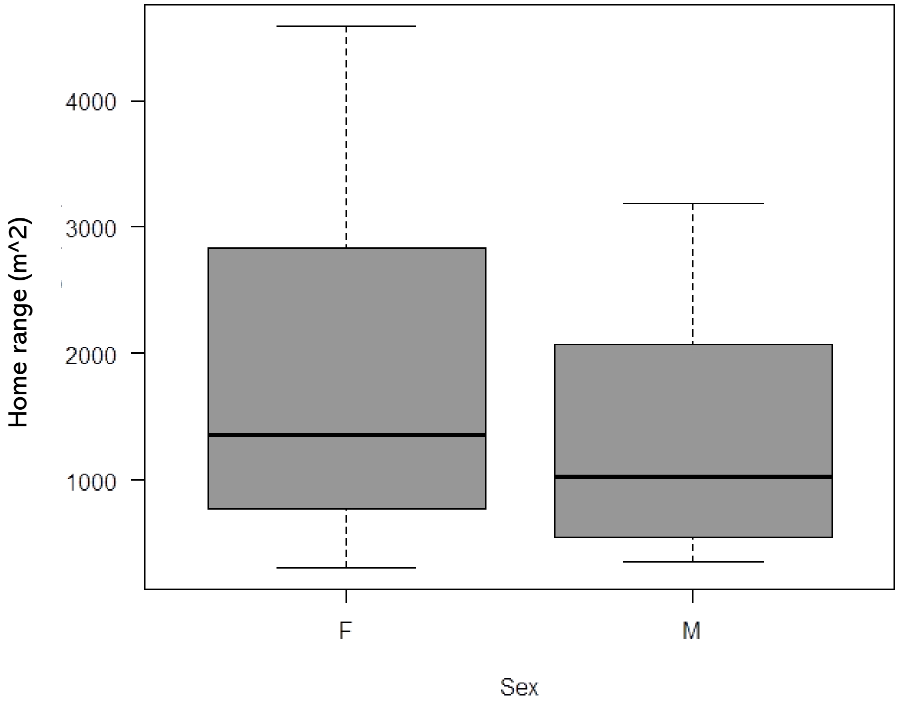


Figure 20: Lizard home ranges in m^2, across sex. Data from multiple years, consult the methods section for procedures in estimating home range from fewer than 24 waypoints.

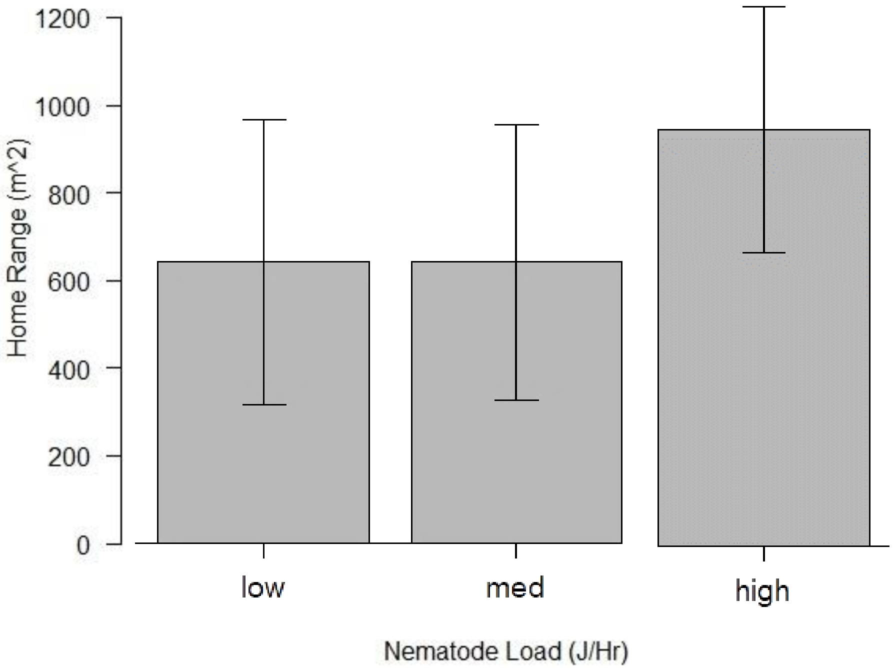


Figure 21: Home range sizes in m^2 (adjusted for body size), across nematode load categories, error bars represent 95% confidence intervals.

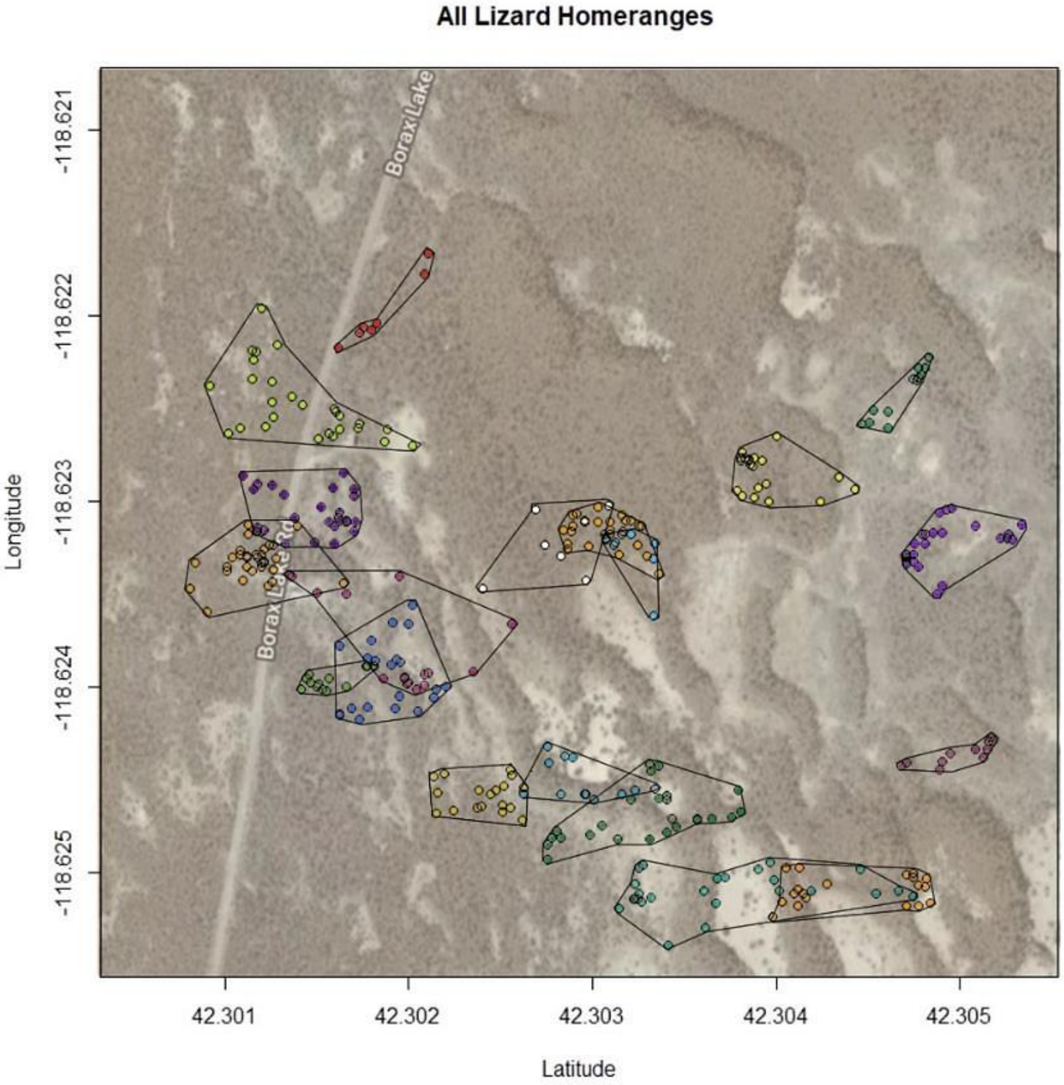


Figure 22: Home range waypoints and minimum convex polygons superimposed over satellite imagery of the study site, individual lizards in different colored waypoints**.** Map generated using Google MyMaps^{TM}.

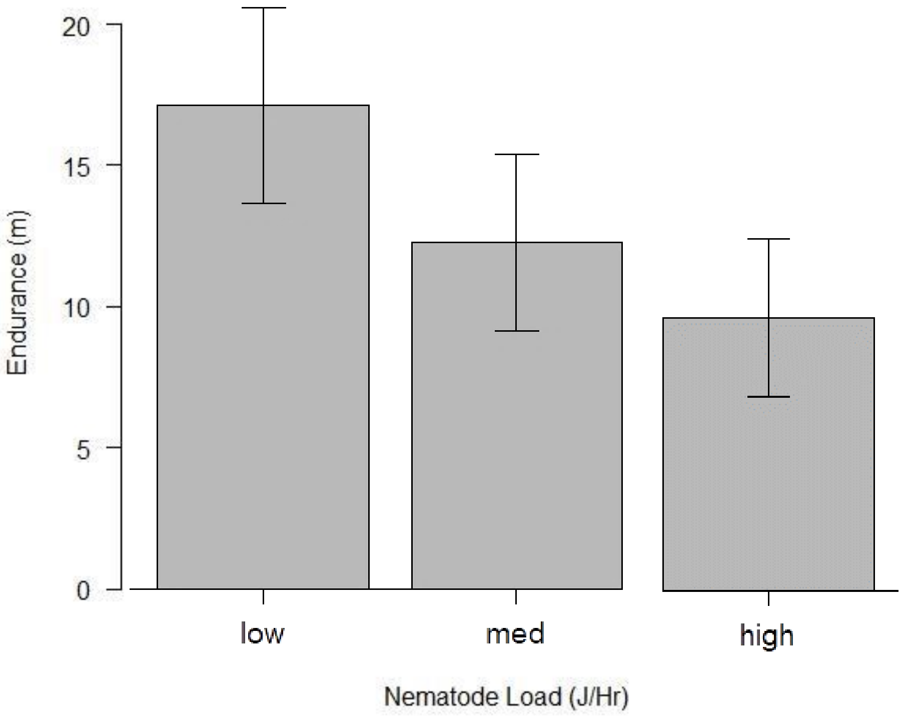


Figure 23: Running endurance of desert horned lizards (adjusted for body size), measured as meters run until exhaustion across nematode load categories. Error bars represent 95% confidence intervals.

Question 2: Body Condition

Females were significantly larger in BCI than males (ANOVA, df=(1,182), F=22.67, $p=3.9x10^{-6}$, $\eta^2=0.111$, female n=91, male n=93, ***Figure 24***), data from 2015-2018. In both sexes, BCI strongly increased with SVL (Type 2 OLS females: p=>0.001, $r^2=0.475$, n=91, males: $p=4.45x10^{-22}$, $r^2=0.637$, n=93). In both sexes, BCI also increased with nematode load (Type 2 OLS females: p=0.059, $r^2=0.165$, n=16, males: p=0.001, $r^2=0.448$, n=18). In females, BCI varied greatly with reproductive state, with females in progressing stages of follicle development also having greater BCI, until becoming post-reproductive and losing much of their mass, (ANOVA, df=(4,179, F=18.847, $p=5.99x10^{-13}$, $\eta^2=0.29$, ***Figure 25***). NR=Non-reproductive, EYF=early yolking follicles, LYF=Late yolking follicles, PR=Post-reproductive. When corrected for the influence of SVL as a covariate, both sexes had significantly heavier BCI at higher nematode loads (ANCOVA females: df=(2,1,12) load p=0.045, svl p=0.0008, n=16**,** males: df=(2,1,14) load p=0.121, svl p=0.0004, ***Figure 26***).

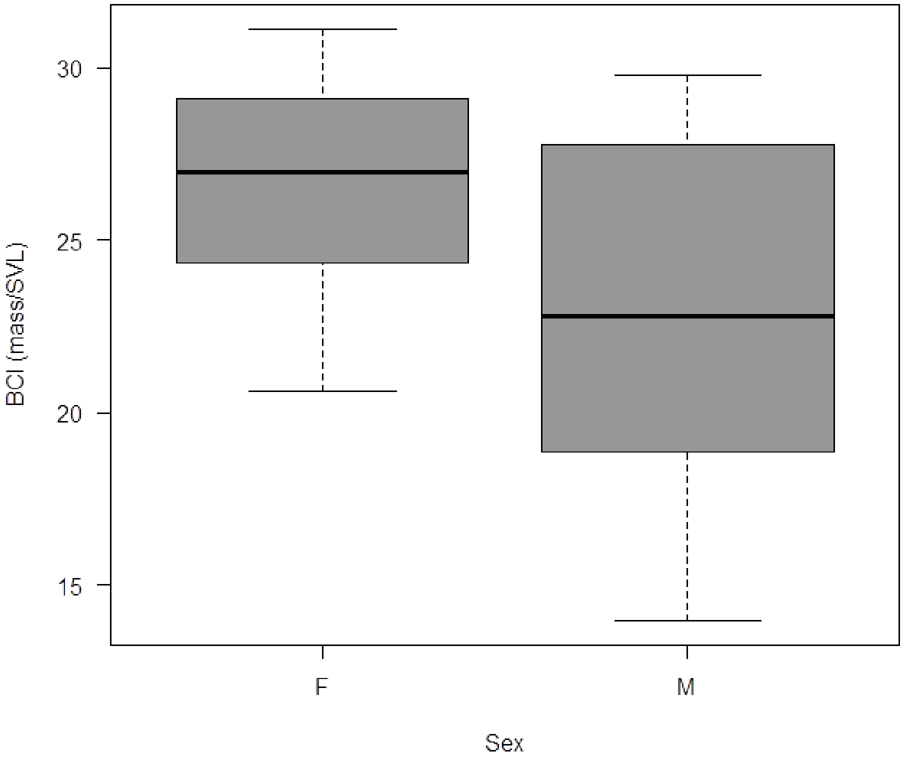


Figure 24: Lizard body condition index (BCI) in mass/snout-vent length in females vs males. Data from 2015-2018.

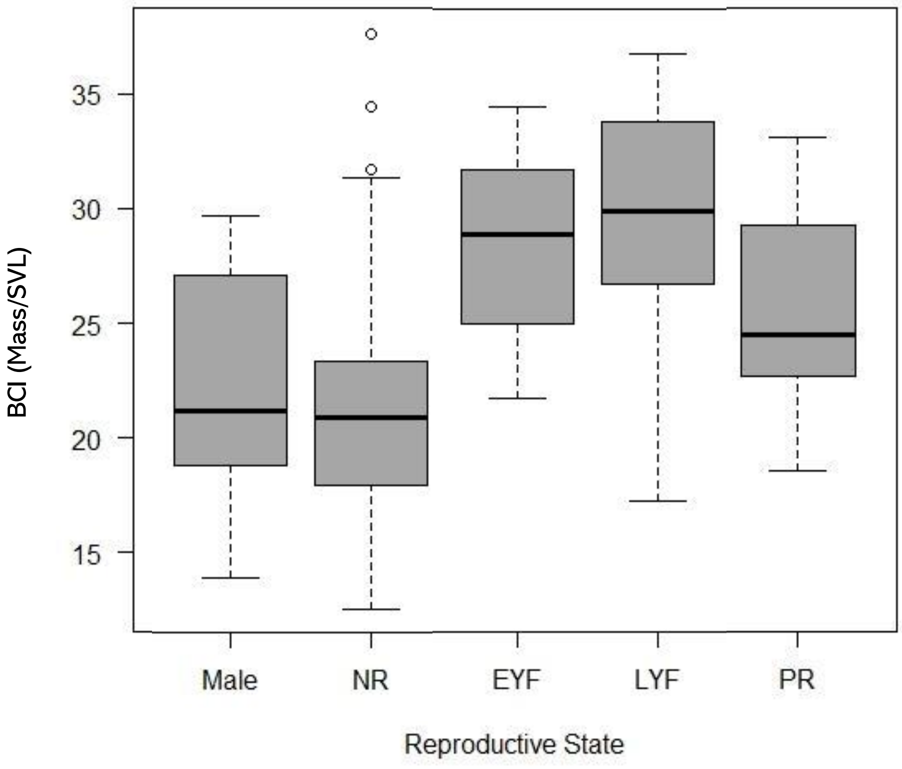


Figure 25: Body condition index in female lizards across reproductive states. Male n=19, NR n=9, EYF n=3, LYF n=6, PR n=3. Body condition data from 2015-2018 Body Condition: Male n=37, NR n=45, EYF n=16, LYF n=18, PR n=19.

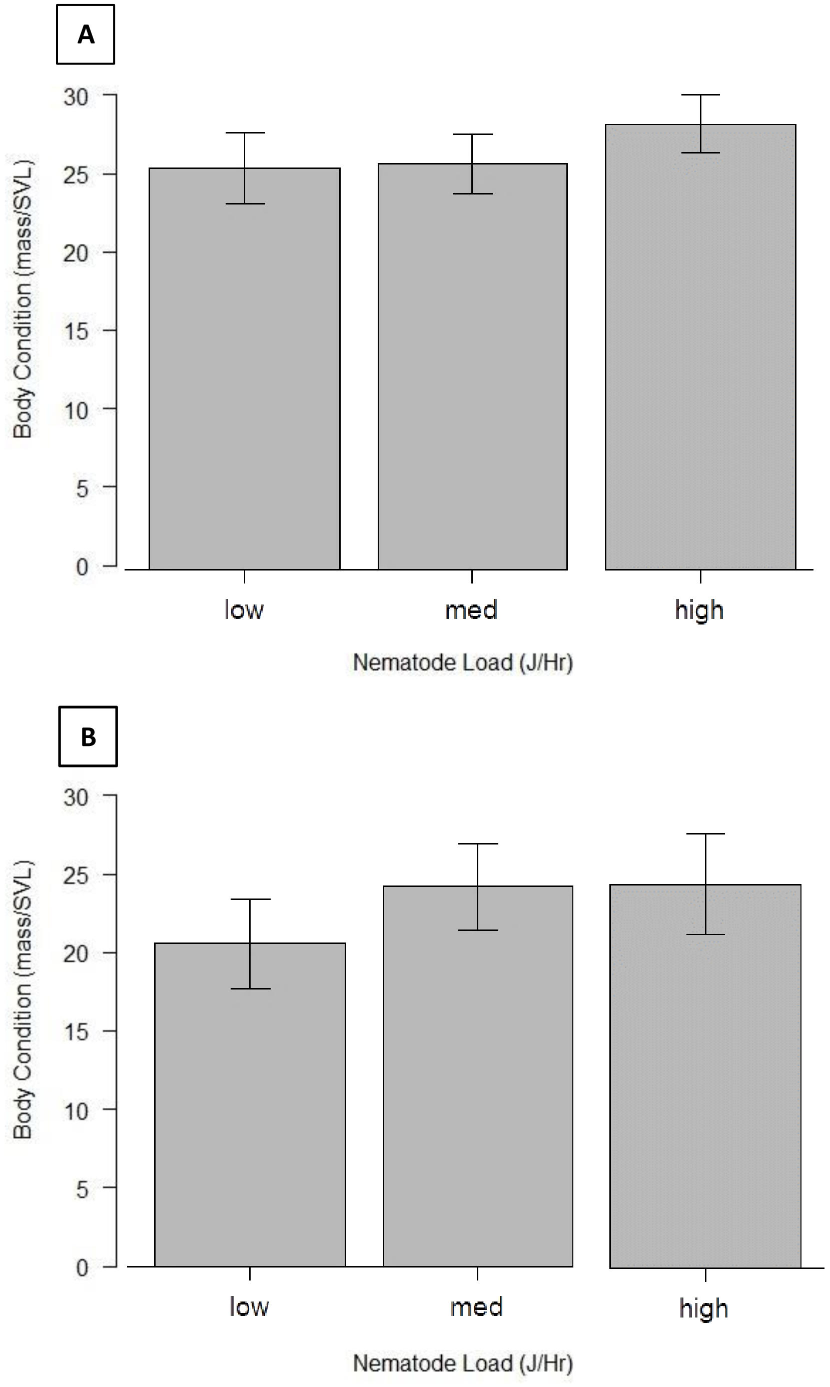


Figure 26: Body condition index (mass per unit length) of **A)** female and **B)** male horned lizards (adjusted for body size) across nematode load categories, error bars represent 95% confidence intervals.

Question 3: Parasites other than *S. phrynosoma*

Ectoparasites

The number of trombiculid mites, the only notable ectoparasite of desert horned lizards, was not different between the sexes (ANOVA, df=(1,38) F=0.102 p=0.7512, females n=21, males n=19). There was no significant direct linear relationship between mites and snout-vent-length (Type 2 OLS p=0.101, r^2=0.044, n=40). Though statistically insignificant when analyzed via regression, lizards seemed much more likely to have an abundance of mites if they also had greater than 2.5 joules/hour of nematode-associated energy expenditure (Type 2 OLS p=0.072, r^2=0.05, n=40, ***Figure 27***). Foraging distance per day was also positively correlated with number of mites with a correlation coefficient of 0.508, (***Figure 28***).

Non-Nematode Endoparasites

The percentage of lizard exhibiting mobile proglottid sections, which indicates infestation by tapeworms (the only major non-nematode gastrointestinal endoparasite of desert horned lizards) is very similar across each nematode load category (44% in low nematode load lizards, n=11, 62% in medium, n=10, and 44% in high, n=10). Additionally, the presence of cestodes seemed to not significantly influence any of my chosen response variables. Though likely not significant, the presence of proglottids may have correlated to number of ants consumed, running endurance and velocity, and blood hematocrit, presence of cestodes n=13, absence n=27 (***Figure 29***).

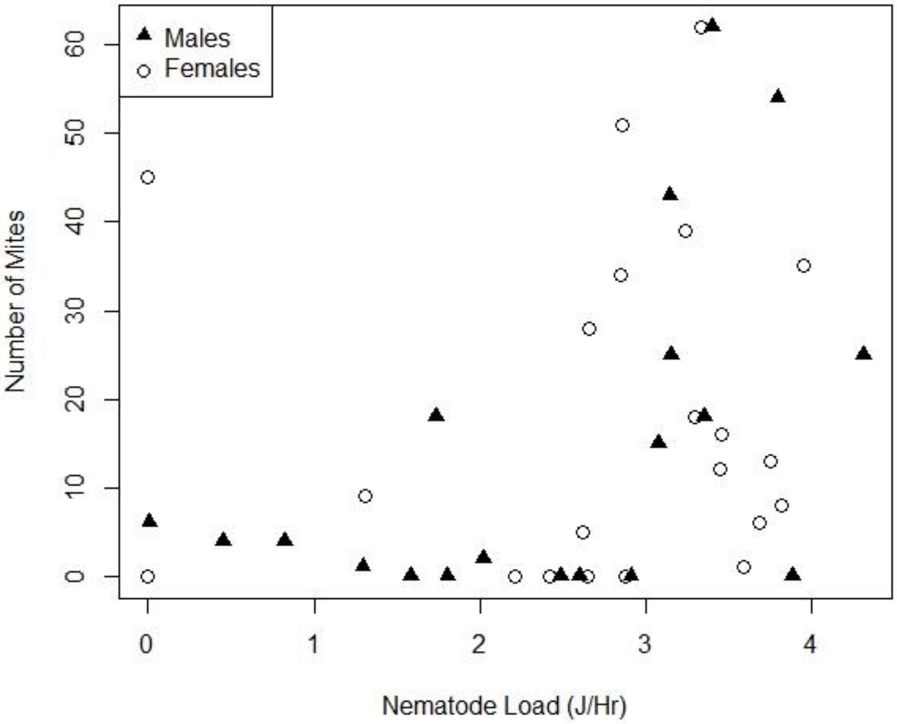


Figure 27: Number of trombiculid mites on lizards with increasing nematode load.

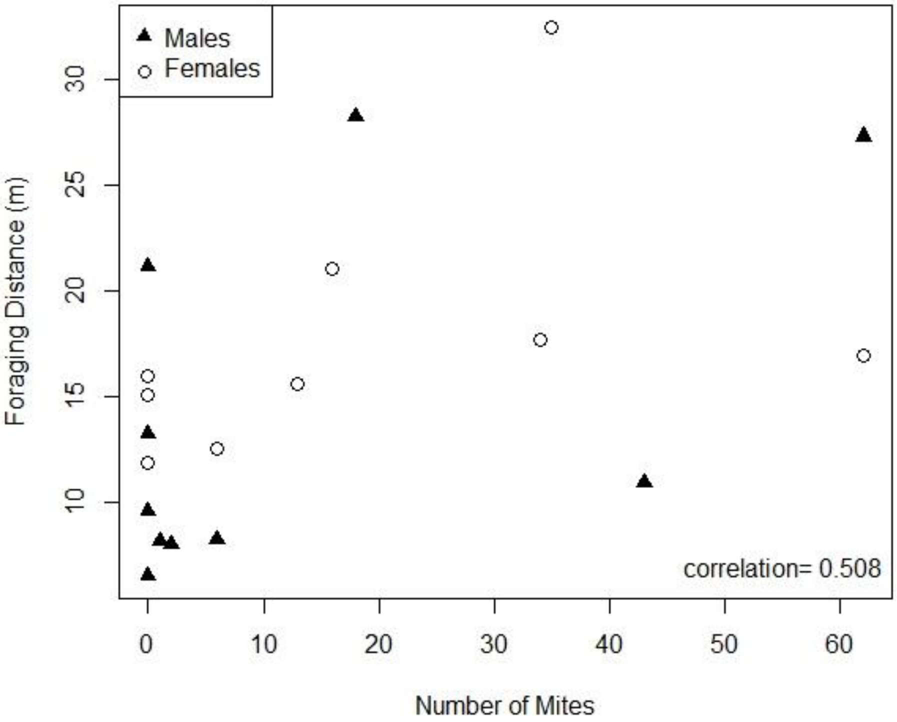


Figure 28: Number of trombiculid mites on male and female lizards potted against increasing foraging distance in meters/day. Males are represented by triangles, females by circles. Females n=9, males n=10. Pearson correlation coefficient is 0.508.

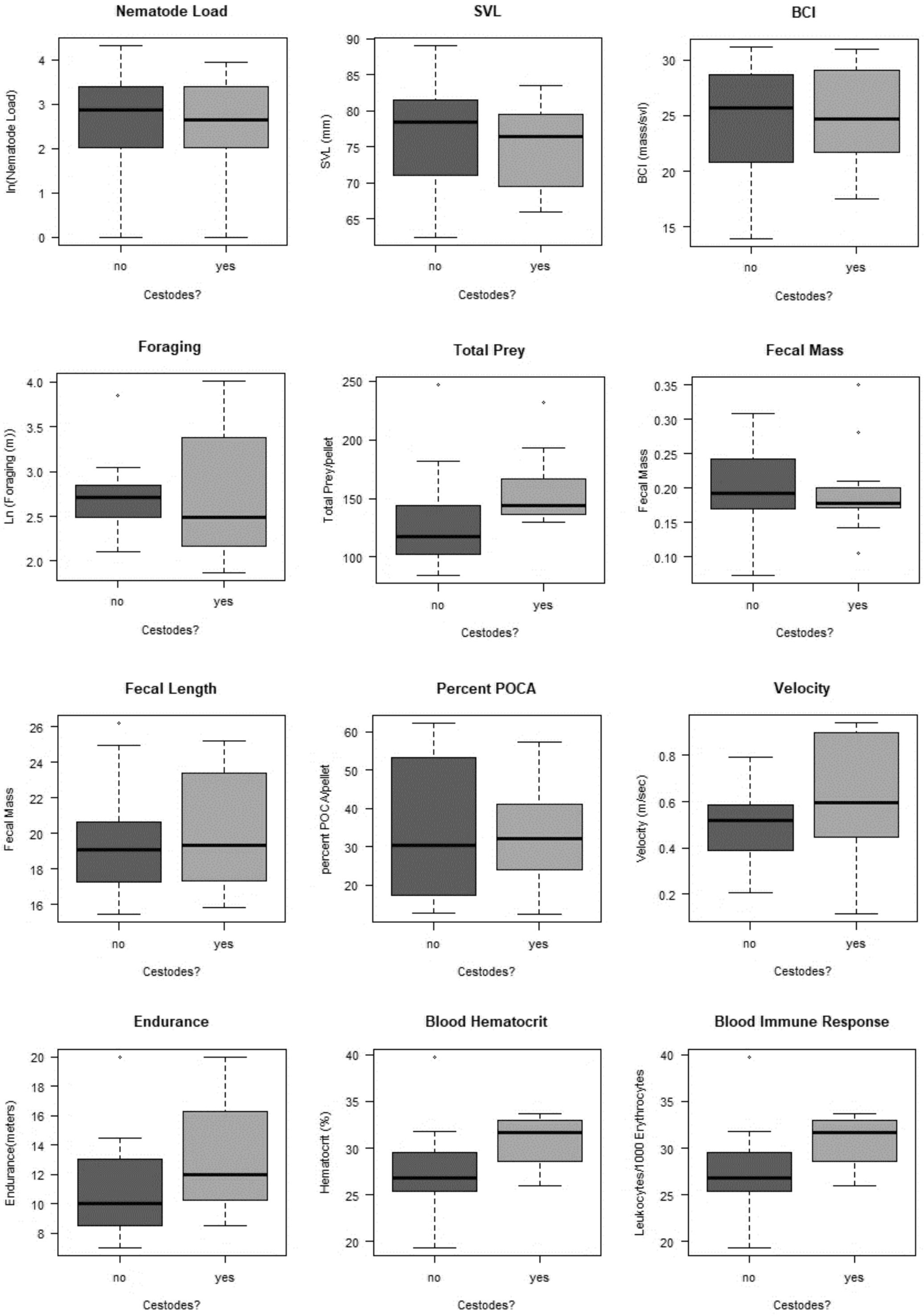


Figure 29: Nematode load, snout-vent length and each other response variable by the presence or absence of mobile tapeworm proglottid sections, here generally termed “cestodes”.

Question 4: Blood Features

Hematocrit

Hematocrit was not different between males and females (ANOVA, df=(1,14), F=0.123, p=0.73, η^2=0.0052, females n=7, males n=9). Hematocrit was not related to SVL (Type II OLS, p=0.297, r^2=0.026, n=14), or nematode load (Type II OLS, p=0.4735, r^2=0.003, n=16). Because hematocrit showed no relationship with SVL, ANCOVA is not necessary.

Blood Immune Response

Blood immune response, measured as the number of immune components (leukocytes) per every 1000 red blood cells (erythrocytes), might have been be lower in females than males, though at the sample size used, there was no significant difference (ANOVA, df=(1,25), F=3.84 p=0.061, η^2=0.34, females n=12, males n=15, ***Figure 30***). Blood immune response showed an inverse relationship with SVL, albeit statistically insignificantly (Type II OLS p=0.0922, r^2=0.075, n=25). Similarly, there was a statistically insignificant inverse relationship of blood immune response with nematode load (Type II OLS p=0.1829, r^2=0.03, n=27, ***Figure 31***). Though there was no significant linear relationship of blood immune response with nematode load, ANOVA was done to examine any differences in blood immune response across the three nematode load categories. ANOVA revealed that lizards of a high nematode load had a significantly lower blood immune response than lizards with a medium or low nematode load (ANOVA, df=(2,24) F=3.6, p=0.0406, η^2=0.23, n=28, ***Figure 32***).

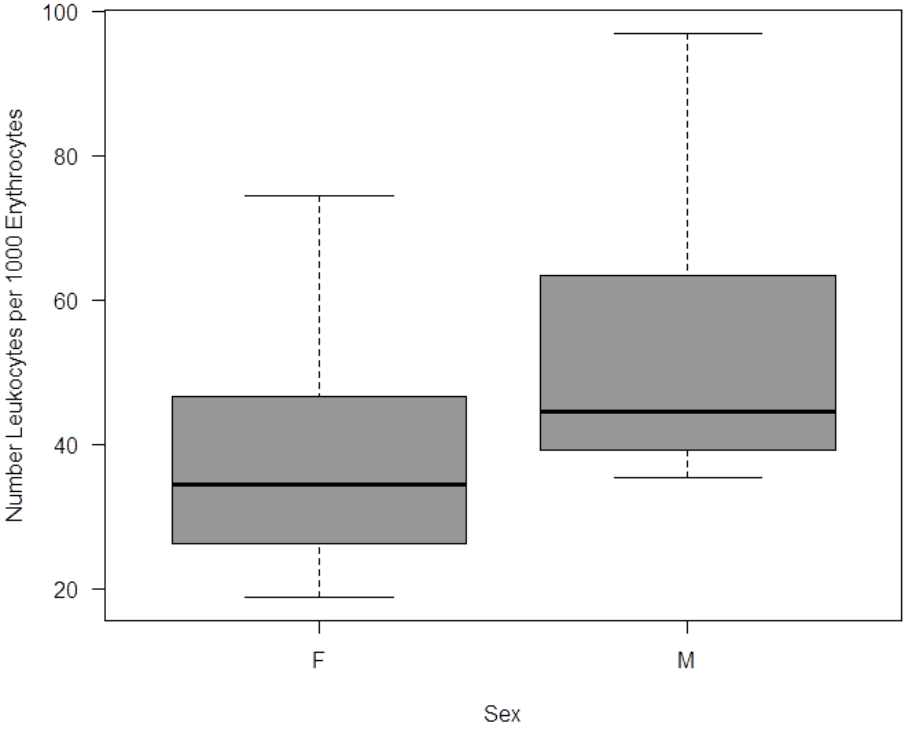


Figure 30: Blood immune response as estimated by number of leukocytes/1000 erythrocytes for male and female horned lizards.

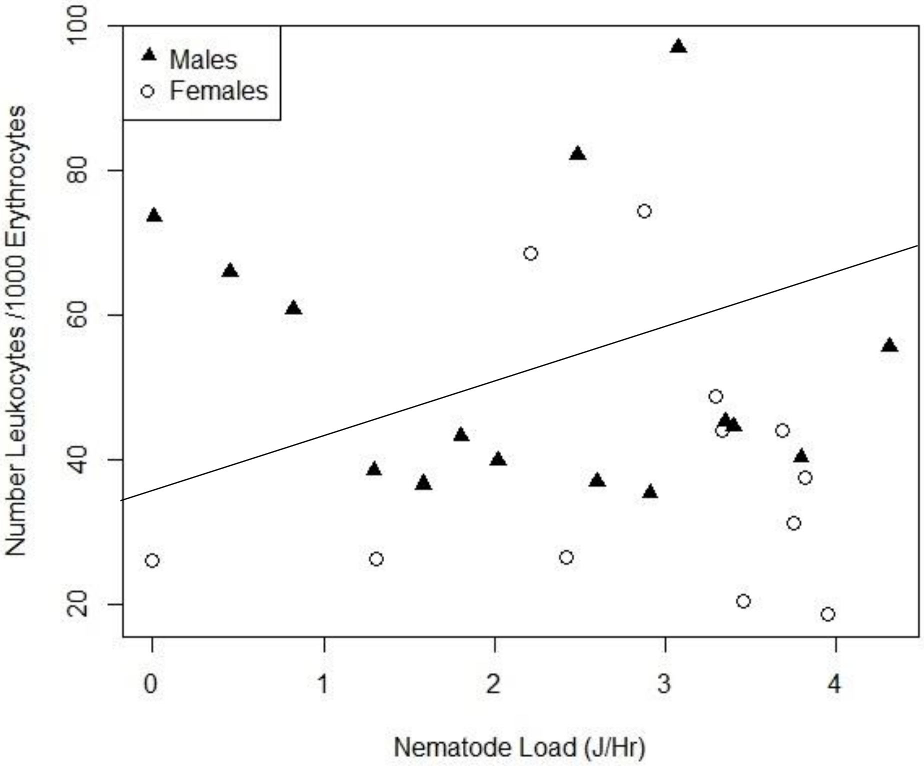


Figure 31: Type II OLS Regression of blood immune response as number of leukocytes/1000 erythrocytes in lizards with increasing nematode load in joules//hr.

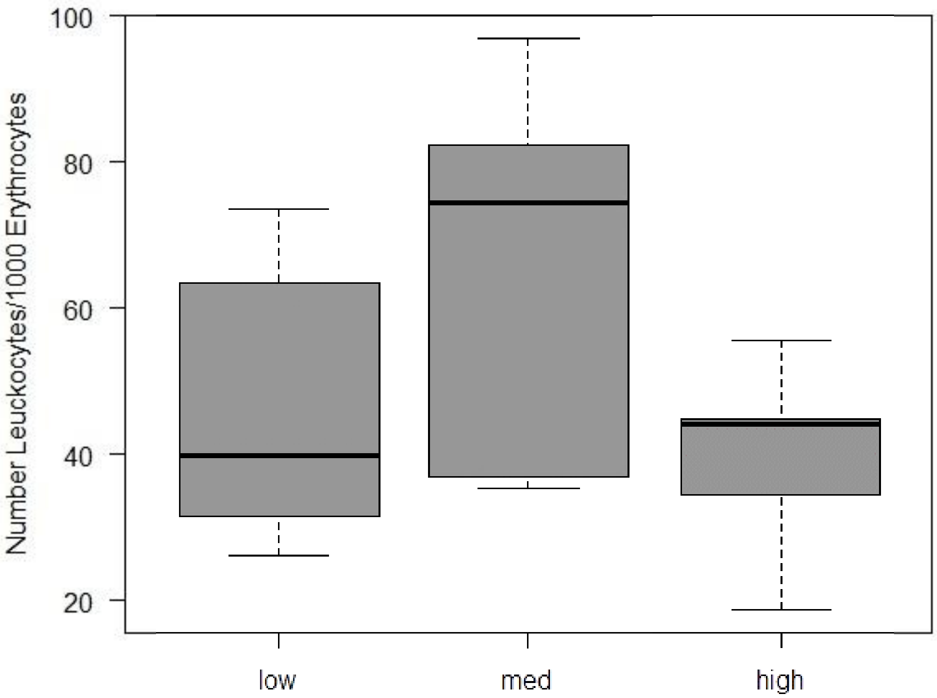


Figure 32: Blood immune response as estimated by number of leukocytes/1000 erythrocytes in lizards with increasing nematode load categories.

Female Reproductive History

Females who were in active reproduction (early or late follicular development and post-reproductive) tend to be significantly larger-bodied than non-reproductive females, though NR females have higher variance in SVL than reproductive females. (ANOVA, df=(4,129), F=20.394, $p=2.68\times10^{-13}$, $\eta^2=0.393$, males n=37, females: NR=45, EYF=16, LYF=18, PR=19, ***Figure 33***). For all females for which body data was taken in 2015-2018 there were 7 females who were recaptured from one year into the next (15-16, 16- 17, or 17-18) and one female captured in 2016 and 2018. ***Table 1*** indicates that 5 lizards (3, 4, 8, 9, 10) grew in SVL and went from non-reproductive to reproductive. Three females were reproductive for two consecutive years of capture (1, 3, 7), but three other adult females (2, 5, 6) were non-reproductive for one of their two captures years.

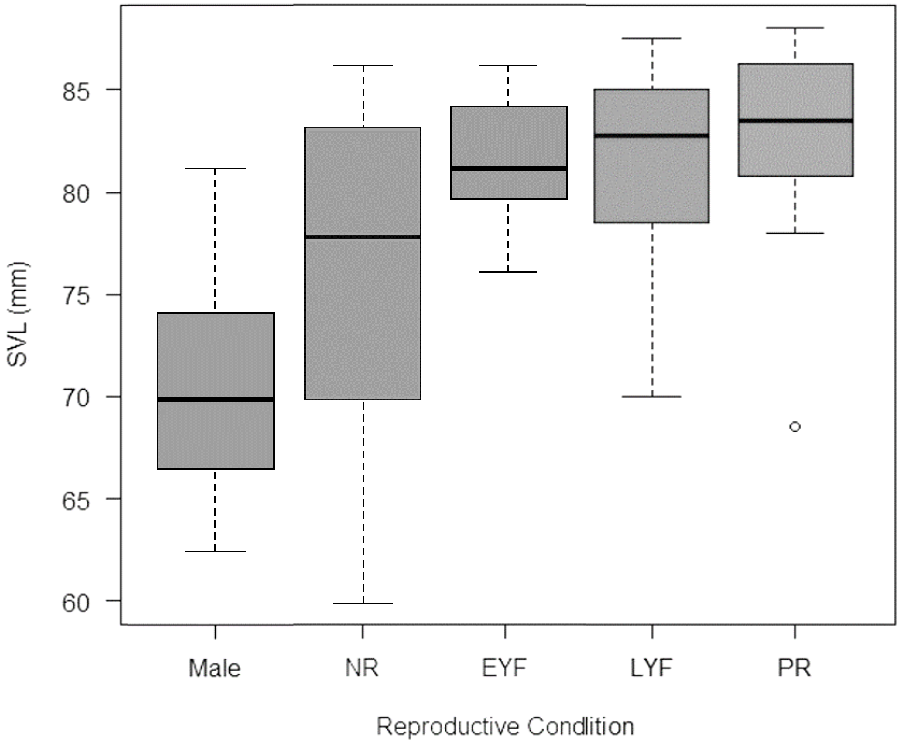


Figure 33: Boxplot of SVL of male lizards and female lizards across reproductive states. Data from 2015-2018 Male n=37. NR n=45, EYF n=16, LYF n=18, PR n=19.

Table 1: Details of all females recaptured from 2015-2018, showing their year(s) captured, development in size and mass, and reproductive state. NR=non-reproductive. EYF=early yolking follicles, LYF=late yolking follicles, PR=post reproductive.

Number	Year	Toe Clips	SVL (mm)	Reproductive State	Mass (g)
1	2015	81220	84.5	LYF	25.22
	2016		86.5	PR	20.81
2	2015	3091218	63.5	NR	8.7
	2016		78.5	NR	17.6
3	2015	3101119	70	NR	15.68
	2017		83	EYF	28.63
	2018		85	EYF	28.33
4	2016	4091216	69.87	NR	12.74
	2017		81.5	LYF	28.42
5	2016	4101516	81.5	LYF	30.53
	2018		83	NR	-
6	2016	5061120	85	NR	27.01
	2017		85	LYF	22.53
7	2017	5071520	70	LYF	12.08
	2018		83.5	PR	22.11
8	2017	5081216	77.7	NR	13.9
	2018		81	EYF	23.1
9	2017	5081318	76	NR	9.55
	2018		81	EYF	21.82
10	2017	5081519	64.5	NR	9.89
	2018		79.5	EYF	24.82

DISCUSSION

Foraging distance moved per day, energetic endurance and body condition index were greater in lizards with longer body lengths, and thus it was relevant to use ANCOVA to reduce the influence of body length as a covariate for these variables. The only significant detriments for lizards at higher nematode loads were significantly reduced energetic endurance and blood immune response. The total number of ants consumed (*i.e.* ants per fecal pellet), the lizards home range size, and their blood hematocrit was not influenced by nematode load, but foraging movement per day and body condition index were positively associated with nematode load. Percentage of POCA out of all ants consumed was significantly higher in females than in males. In male lizards, percent POCA consumed did not change with nematode load, but in female lizards, percent POCA consumed was negatively associated with increasing nematode load.

The costs of parasitism

Two primary fitness challenges to the host may shape the relationship with their parasites over evolutionary time: the inherent level of detriment to the host from energy loss to the parasite, and the dampening of adverse effects over time due to the mutual benefits of host health (Wood & Johnson, 2015). Hypotheses for this study predicted that although nematode infestation in horned lizards may be mild enough for both populations of these co-evolved species to persist, horned lizards will suffer at higher nematode loads given the limited food availability typical of desert biomes.

A parasite's effect on its host depends upon host and parasite physiology, the nutrition available through the host (*e.g.*, parasitic consumption of host's stomach contents or host's tissues), and the host's ability to resist the parasite. It is well known that in mammals, gastrointestinal nematode parasites are strongly detrimental. In both livestock and wild mammals, nematode infestations generally result in malnutrition, poor body condition, and are often fatal if left untreated (Mavrot *et al.* 2015, Stien *et al.* 2002). High nutrient availability and spatial proximity among hosts promote the persistence of the parasite population even if the host eventually is killed (Arneberg, *et al.* 1998). In the case of nematodes and horned lizards, the small body size and relatively short life span limits the parasite abundance within the host, and relatively low population densities and low spatial proximities of lizards as well as the limited nutrient availability to hosts and parasites might have led to a more steady, low-impact parasite load that optimizes fitness of parasites and hosts at only a moderate cost to the host.

The coevolution of host and parasite may be why nematode infestation in horned lizards was more benign than hypothesized (relatively few response variables showed a negative association with nematode load and foraging, and body condition index actually showed a positive relationship). Desert horned lizards, as dietary specialists in a nutrient-poor environment, must eat the largest, most available ant-*Pogonomyrmex californicus*-making these ants an ideal intermediate host for the nematodes. Due to this quasi-obligate feeding of the infective ant species, most individual lizards become parasitized, creating selection pressure to reduce adverse effects of the parasite upon its host. Out of 40 lizards captured in summer 2018, only two exhibited no sign of nematodes. The following sections will discuss the metrics first demonstrating a direct, then neutral, and then inverse relationship with nematode load.

I hypothesized that increased nematode load would increase energetic needs and thus foraging related metrics, but at high nematode load could cause host illness and a reduction in foraging metrics and body condition. I also hypothesized that abundance of other parasites may be higher at increased nematode loads, possibly due to nematode-mediated illness. These hypotheses were partially supported. Among the nematode loads observed in 2018, horned lizards with higher nematode loads foraged for greater distances and had a heavier BCI compared to lizards with lower nematode loads, ***(Figures 12, 26.)*** These increased feeding-and-assimilation related metrics could be related to the ability of larger-bodied, more robust lizards to accommodate a larger nematode load or to the possibility of increased parasite load eliciting a compensatory effect to allow lizards to sustain both themselves and their parasites.

Even when the analysis is adjusted to correct the effects of body size, desert horned lizards infested with the highest nematode load moved further during their daily foraging than lizards infested with a lower nematode load (***Figure 12***). Although foraging modes and feeding rates are known to influence parasite load and diversity (Sluys *et al.*, 1997), little work has focused on how parasite load influences foraging effort and net rate of energy intake**.** The current understanding of parasite avoidance is limited in both lizards as a study organism and with helminths as a parasite type, because most cited studies focus on mammals, birds, and either enteric bacterial pathogens or bloodborne parasites. Foraging may be impacted by parasite avoidance behaviors; for example, many species of mammals use olfaction to detect food contaminated with conspecific feces, thereby avoiding enteric parasites and pathogens (Sarabian and Balais, 2018). Unlike the pre-ingestion behavior of mammals, the increased foraging effort by horned lizards enduring nematode infestation may be a post-infestation compensatory effect. Given that a typical rate of infestation doesn't incapacitate the lizard host, it may be inferred that the presence of the nematode as internal competitor causes the lizard to forage more to feed both itself and its parasite and forage for greater distances to access enough ant colonies to do so.

Lizards are known to increase foraging effort to meet energetic challenges such as reproduction (Schwarzkopf, 1996) and tail loss (Martin & Salvador, 1997) thus, it follows that increased energy costs of parasitism by nematodes could lead to more intensive foraging by horned lizards. Although studies on parasite-mediated compensatory behavior in lizards are lacking, the phenomenon of parental compensation for parasite load is demonstrated in many species of birds. Blue tits, for example, increased the rate of food provisioning to bot-fly infected nestlings by 29% compared to unparasitized chicks (Tripet & Richner, 1997).

The concept of behavioral compensation by host in response to parasites may also be the cause for lizards with more nematodes show significantly higher BCI relative to lizards with fewer nematodes, even when adjusted for variations in body size. (***Figure 26***). Lafferty's model (1992) suggests that the energy gained by increased prey consumption and by eating more nutritious prey (even if that prey is a transmitter of parasites) outweighs the energy lost back to those parasites. In *Passalus* beetles

(*Coleoptera: Passalidae*) parasitized with a nematode, parasitized beetles had a significantly higher BCI and larger body size (Cox and Davis, 2013), and were more efficient at processing wood (Davis and Prouty, 2019) than their unparasitized conspecifics. Gastrointestinal helminths of the genera *Parapharyngodon and Physaloptera,* at infestations observed in a study of lacertid lizards, did not affect BCI in either sex, even in reproductive females (Galdino *et al*., 2014). In the case of desert horned lizards, if lizards are to grow or to invest energy into reproduction, and if they are to eat more ants (or higher quality ants) and thus accumulate more nematodes with minimal harm, then larger fat pads and higher BCI could be expected in more heavily parasitized lizards.

Though it did not meet the parametric assumption of normality, the number of trombiculid mites appeared to positively correlate with nematode load (***Figure 27***). This result is inconsistent with the alternate hypotheses in this study, where any positive correlation between nematode load and abundance of other parasites was hypothesized to be byproduct of nematode-mediated illness and/or reduced resistance to mites. Instead, I attribute the increased mite prevalence to the increased foraging distance presented in lizards with greater nematode loads. Foraging gives the opportunity for lizards to encounter more mites, and as such it is understandable that foraging distance per day is positively related to number of mites (***Figure 28***). I also hypothesized that the influence of nematode parasitism on the greater host community would include influence on abundance blood-based parasites found within desert horned lizards (Babero & Kay, 2015). This study was unable to support or reject this hypothesis, as the methods used for immune determination were not able to detect any bloodborne parasites or pathogens. In this case, non-detection does not satisfactorily indicate non-presence, considering Barbero & Kay, (2015), detected 5 types of bloodborne protozoa in desert horned lizards.

Understandably, not all metrics of horned lizard health were related to nematode load. I hypothesized that home range and hematocrit would not be affected by nematode load, that velocity would be negatively affected, that number of prey consumed would increase proportionally with foraging metrics. All these metrics were unaffected by nematode load, supporting the hypotheses for home range and hematocrit, and not supporting the hypotheses for velocity and number of ants (***Table 2***). Because parasitism has not been shown to influence home range in other lizards (Bull, Burzacott, 1993 & Schall, Houle, 1992), it is likely that any apparent relationship of home range size is due to a correlation with body size. Consistent with the results of this study, home range size in lizards is directly related to body size, and both factors are commensurate with a lizard's energy needs (Gad & Garland, 2002, Christian & Waldschmidt, 1984). Increasing home range size as a response to energy demand may explain the broader home range in females compared to males, (***Figure 20***). Larger horned lizards are expected to require more visits to more ant colonies, hence requiring larger home ranges to increase the chances of finding a surface-active ant colony, and thus giving larger lizards the opportunity to encounter more nematode-infected ants and accumulate more nematodes.

My finding that nematode load was not associated with maximum short-sprint velocity in these putatively healthy horned lizards corroborates a study in sand lizards (*Lacertidae*), showing no link between parasite load and sprint speed (Ekner-Grzyb *et al.*, 2013). Sprint speed is primarily influenced by morphological traits, such as hindlimb length (Bonine and Garland, 1999). Moreover, in lizards there is no apparent trade-off between sprint speed and endurance (Albuquerque, Bonine, & Garland, 2015), hence sprint speed is not expected to be related to the energetic costs of nematode load.

Hematocrit, the volumetric proportion of cells to blood plasma, may be an indicator of the oxygen-carrying capacity of the blood and of hydration state. Hematocrit of birds and lizards can be reduced by blood-consuming ectoparasites such as ticks (Wanless, Barton and Harris, 1997, Dunlap and Mathies, 1993), but in the case of parasites that do not consume blood—such as filarial worms infesting frilled lizards— there is no effect of parasite load on hematocrit (Christian and Bedford, 1995). Similarly, because *S. phrynosoma* does not consume lizard blood, it is reasonable to expect that nematode infestation at the levels found in this study does not affect horned lizard hematocrit.

Although the effect of nematodes on horned lizards appears to be modest, there were some fitness-related metrics which were inversely related to nematode load. I hypothesized that POCA consumption, fecal mass and length, and blood immune response would decrease with increasing nematode loads. These hypotheses were mostly supported, but there were some sex-specific differences in trends that I did not anticipate. Running endurance and blood immune response was lower in lizards at the higher nematode loads (***Figures 23, 32***). Females with a larger nematode load showed reduced POCA consumption and reduced fecal length and mass compared to females with smaller nematode loads (***Figures 18, 14***).

Parasitism has been shown to negatively affect exercise effort or capacity in variety of taxa, from flight endurance of invertebrates (Luong *et al.*, 2015), hopping stamina of toads (Goater, Semlitsch & Bernasconi, 1993), and running endurance of lizards (Main & Bull, 2000, Clobert *et al.*, 2000). Similarly, running endurance was significantly reduced in horned lizards with greater nematode load (***Figure 23***); but the mechanism for this reduced endurance is unknown. For example, we do not know whether motivation to run is reduced—as related to malaise—or whether ability to run is related to physiological fatigue. As parasitism in general is linked to stress response (*i.e.* heightened glucocorticoids) albeit in mammals (Defoilie, Merkling & Fichtel, 2020) and sickness behaviors such as lethargy is a common pathogen response (Adelman & Martin, 2009), the reduction in endurance at high nematode loads could be attributed to lizards experiencing malaise.

One of the most interesting results of this study for the readers may be that lizards with a higher nematode load had a significantly reduced blood immune response compared to lizards of medium and low nematode loads when examined vis ANCOVA, (***Figure 32***). Immune responses were not significantly different in lizards with low and medium nematode loads, with a combined average of around 60 leukocytes per 1000 erythrocytes. However, lizards with high nematode load had significantly reduced blood immune response, averaging 35 leukocytes per 1000 erythrocytes. It is established that in large mammals and in humans, endoparasite infestations can have an immunomodulatory effect, likely mediated by the presence of immune-suppressive molecules on the surface of the parasites, presumably as a protection for the parasite against host resistance (Maizels & Philipp, 1982, Loukas & Prociv, 2001). Studies on these interactions in reptiles, especially lizards, are extremely limited. It seems reasonable to infer that nematode-mediated immune suppression may be the cause of the reduced number of leukocytes in lizards with more nematodes. Furthermore, it is well known that maintaining immunocompetence is costly and thus must exist in a trade-off with other elements of fitness (Schmid-Hempel & Ebert, 2003; Uller, Isaksson, & Olsson, 2006). Thus, larger, nematode-laden lizards may de-emphasize immunocompetence in favor of maintaining body condition and reproductive ability. Tentatively corroborating the hypothesis of costly immunocompetence is the observation that female lizards have a reduced blood immune response at around 35 leukocytes/1000 erythrocytes compared to males at 45 leukocytes/1000 erythrocytes, (***Figure 30***).

Even though nematode load did increase foraging distance, there was no association between nematode load and the number of prey consumed. As these horned lizards are both nutrient-limited by their prey availability and limited in their foraging time by high daily temperatures, they are obligated to use the discrete energy needed to catch each prey item to choose the highest-energy containing ant species available rather than focusing on catching a large number of prey, consistent with findings in other lizard taxa (Paulissen 1987; Stamps, Tanaka & Krishnan 1981; Suarez, Richmond & Case, 2000). It may be inferred that because male horned lizards consume fewer POCA in proportion to their abundance than females (***Figure 19***), prey choice and preference for POCA are based on the context of dimorphic reproductive energy demands.

I hypothesized that as female horned lizards endure substantially higher energetic investment in reproduction than males (Sherbrooke, 2003), female horned lizards will be more severely affected by nematode-mediated malaise than similarly infected males. I also hypothesized that females would demonstrate increased feeding and foraging related metrics compared to males. This hypothesis was largely unsupported, because the largest difference between sexes is in dietary preferences. In female horned lizards, POCA consumption decreases with increasing nematode load, (***Figure 18***). The decrease in POCA consumption shows a mirrored decrease with fecal mass and length, likely because larger POCA exoskeletons form a proportionally heavier and longer fecal pellet, (***Figure 13***). Because horned lizards presumably have an obligation to consume comparatively high-energy packets of food such as POCA at comparatively available colonies, the observation that females with high nematode loads decrease POCA consumption despite being especially energetically challenged may be due to parasite avoidance. This phenomenon will be discussed in the following sections, as POCA consumption defines the paradoxical relationship of the ants to the lizard, that these ants are both an essential source of nutrition for these horned lizards, and the source of their parasites.

A critical stipulation to interpreting these data is that the effects of nematode observed here are fundamentally related to the range of nematode load I was able to capture. While the range of nematode load across the 2018 sampling of lizards seemed reasonably distributed, spatial and temporal variations in climate, ecology, specific diet, and systemic nematode densities throughout horned lizards range indicate there is likely nematode load range variation which this study may not have captured. The lack of strong detrimental effects at “high” nematodes found in these results may be because the range at which those effects would be seen was not present in the horned lizards available for study. Typical ranges for number of nematodes (not nematode load) in this study were generally 30-150 worms, more consistent with Sherbrooke (2003) while levels described in Babero & Kay, (2015) were often in the hundreds of worms. Though this stipulation exists, this study still provides insight into the pathology of nematode infestations and the forces that determine its severity.

The parasite-host relationship

POCA are the most available large ant to horned lizards in the Great Basin Desert scrub. Revisiting our results on prey choice vs. prey availability: Adult desert horned lizards primarily eat the four most common, large ant species among the 20 ant species caught in pitfall traps on site: *Pogonomyrmex californicus*, *Myrmecocystus kennedyi*, *Crematogaster mormonum*, and *Camponotus hyatti*. These four species comprise about 60% of the ant availability and 95% of the lizards' diet (***Figures 19***). Diet preferences between males and females are almost identical, with the exception that for primary prey, females prefer POCA while males prefer *Myrmecocystus kennedyi* (MYKE), each sex consuming their primary prey choice as around 60% of their diet. Because MYKE are both less available (***Figures 19, 40***) and smaller than POCA (Ha, 2010), male preference for MYKE goes against the predictions of foraging theory, which assumes the net rate of energy intake as currency. For male lizards, perhaps the reduced fitness associated with parasitism outweighs the benefits of POCA consumption.

Considering that lizards with more nematodes forage for greater distances (***Figure 12***) yet don't have a greater number of ants represented per fecal pellet (***Table 4***), nematode load seems to influence prey quality (*i.e.,* prey choice) more than prey quantity. Male lizards don't change their diet based on nematode load, but female lizards vary their dietary preferences depending on nematode load and reproductive state, indicating a potential trade-off in near-term benefit of energy versus longer-term costs of parasitism. Females must either consume the largest and most energetically available prey and maintain well-being during high costs of reproduction one season at the cost of greater parasitism in the future, or avoid POCA consumption and thus reduced future parasite loads but endure a lower rate of energy intake in the present. This tradeoff reveals the intriguingly complex nature of host-parasite dynamics and the fitness-related challenges and opportunities that shape the evolutionary relationship of horned lizard and nematodes.

In a seasonally restricted, short-lived lizard species such as desert horned lizards, the defining energetic expense of their active season is successful reproduction. Males tend to increase movements during the reproductive season and put energy into increased sperm production, albeit their reproductive energy investment is mild compared to females. Most lizard species females invest 10-15% of their body mass into eggs, but female horned lizards invest as much as 35% (Pianka & Parker, 1975). Additionally, females commit to egg production once within vitellogenesis, they do not reabsorb yolking follicles even if stressed energetically (Sherbrooke, 2003). This intense energy expenditure prioritizes large clutches of eggs over females, who are entirely emaciated post-oviposit. For example, during summer 2018, I observed a plump 28-gram, oviductal female who embarked on a four-day egg-laying foray and returned to her home range emaciated, weighing only 15 grams. Based on records of several radio-tracked females in 12 summers of field-research, Dr. Anderson has observed several females who died during or immediately after oviposition. Referring to the body data from 2015-2018, some females of reproductive body size were non-reproductive during the height of the reproductive season (***Table 1***), and the SVL of non-reproductive females has high variance and is not significantly different from reproductive females (***Figure 33)***. Thus, it is reasonable to infer that the energetic cost of a single-reproductive event may be so high that females may not reproduce every year. Given this reoccurring energetic challenge for reproduction in short activity seasons (May to August), the added costs of a heavy nematode load may be of increased relevance to female lizards during the reproductive season.

Like reproductive energy expense, nematode-associated energy expense must also demonstrate seasonal variation depending on lizard activity, food availability, and environmental conditions. As the Alvord basin sustains sub-freezing and freezing temperatures from October-April**,** ectotherms like horned lizards brumate during these winter months and as many days in the months of May and September are also too cool for lizard activity, their activity season is largely restricted to the central 3 months of summer. The consistent presence of juvenile nematode in the lizards throughout the activity season suggests that individual nematodes may be present within the lizards for more than one activity season, with the most and largest nematodes occurring during the height of food availability and reproductive activity in June and July (Hilsinger, Anderson, & Nayduch 2011). Considering female reproductive investment likely varies year-to-year, it is also reasonable that the interaction of varying reproductive investment, nematode load intensity, food availability and drought may critically affect survival of female horned lizards in their reproductive season.

Because the brunt of reproductive costs falls on the females, it is no surprise that this is where we see the most sophisticated relationship between lizards, their food, and their parasites. POCA represent about half the diet of female lizards, and around one-fourth the diet of male lizards, (***Figure 19***). Females are presumed to be obligate consumers of POCA to meet their energy demands, especially during reproduction. This is corroborated by the observation that females with early or late-yolking follicles consume POCA as 50-60% of their diet, while non-reproductive females are more similar to males, in that POCA comprise only about 30% of their diet (***Figure 19***). If nematode load were of no consequence, then the lizards with the highest nematode loads would continue consuming the mostly POCA. However, we observe the opposite. Female lizards with the lowest nematode load consume the most POCA, while lizards with medium and high nematode load consume fewer POCA (***Figure 18***). This phenomenon indicates the energetic and well-being dilemma females face—balancing immediate energy needs with the future costs of parasite load.

Consumption of POCA by females must thus depend on the intersection of the female's present nematode load and her reproductive energy expenses. There are four possible scenarios here: at low nematode load and low reproductive costs, females are not energetically challenged and can eat POCA indiscriminately. At low nematode load and high reproductive costs, females must eat a high proportion of POCA to meet their energy demands. At high nematode load and low reproductive costs, females can avoid further parasitism by avoiding POCA as they are less necessary energetically. Finally, at high nematode load and high reproductive costs, females likely eat POCA to meet their energy needs, risking emaciation due to the intersection of the costs of nematode load and egg development (***Figure 33***).

It is reasonable to infer that balancing the energy benefit of immediate POCA consumption versus energy later lost to parasitism may be the applicable fitness tradeoff in the coevolution of horned lizards and nematodes. Successful reproduction is paramount in this short-lived species, and abundant high-energy packets of food such as POCA are necessary to achieve the high clutch sizes necessary to the lizards reproductive success. If females were not obligated to consume POCA during the reproductive season, MYKE would likely be the primary dietary choice of all horned lizards, not just males, and nematode infestation would be less common throughout the lizard population. Thus, it is expected that there is a fitness benefit for female horned lizards to endure future nematode infestation so that they can fully utilize POCA as a food source for current needs. Conversely, because peak POCA consumption and nematode loads co-occur during the reproductive season, there is a fitness benefit for nematodes to be mild enough in pathology that females neither avoid POCA nor forego reproduction. The timing of maturation and emergence of nematodes may oscillate such that nematode load and reproductive investment avoid simultaneous peaks and favor successful host reproduction. Overall, the costs to parasitism and the selection pressure to achieve consequence-free parasitism results in a fascinating energetic, physiological, and ecological dynamic of which should compel abundant future research.

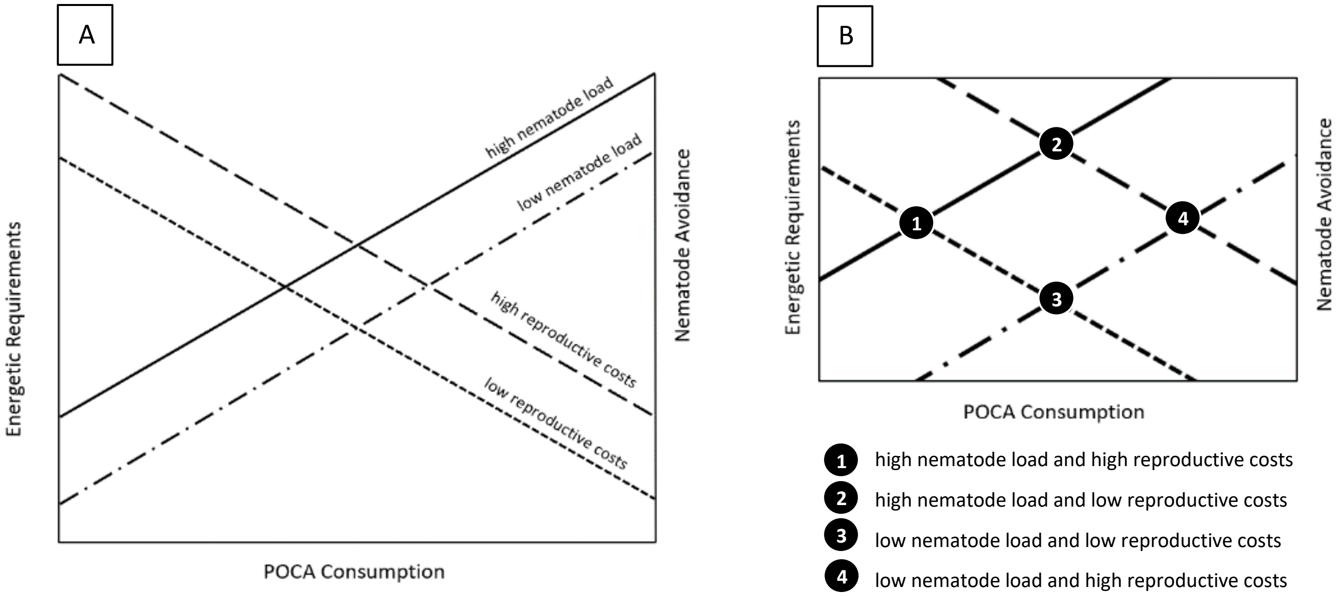


Figure 33: **A)** the effects of energetic need and parasite avoidance on horned lizard POCA consumption in the context of reproductive costs (high or low) and nematode load (high or low). **B)** an emphasized view of the intersection between nematode load and reproductive costs, and their influence on POCA consumption.

Conclusions

Essentially every desert horned lizard living in the challenging environment of the Alvord Desert of southeastern Oregon exists with some number of gastrointestinal nematodes, *Skrjabinoptera phrynosoma,* in its stomach, reproducing and feeding on the lizard's stomach contents. The range of nematode load seen in this study—represented here in the summed joules/hour of resting metabolic rate of all the nematodes within a lizard—was more benign to that lizards' ability to survive, thrive and reproduce than was hypothesized. Further research is needed to elucidate the ability of horned lizards to mitigate infestations as well as the parasitic pathology of the nematodes themselves.

Larger lizards carried greater nematode loads and had larger home range sizes, foraging distances, and reduced endurance, demonstrating the need to include snout-vent-length as a covariate in all analyses. The inclusion of this covariate reveals the real costs of parasitism in lizards of all body sizes. Greater nematode load does show some exercise and energetic consequences for lizards, as lizards with more nematodes can't run for as long and evidently must compensate for their nematode load by moving further throughout the day to find more POCA to consume. Additionally, possibly nematode-mediated reduction in blood immune response may have implications for abundance of other parasites such as trombiculid mites, though results were not conclusive.

Despite the adverse effects of nematode load, lizards with a greater nematode load were still able to forage for longer distances and develop a heavier mass per unit length than conspecifics with a lower nematode load, perhaps due to compensatory effects and the mild pathology in this coevolved host-parasite system. Nematodes are abundant in the system because their intermediate host, the harvester ant *Pogonomyrmex californicus,* is a critically important food source, especially for female horned lizards during egg development. These parasites represent simply another energetic challenge—one that lizards can mitigate with behavior and energy budgeting. The observation that horned lizards can sustain a large parasite population in its stomach on a nutritionally poor diet while meeting the demands for maintenance metabolism and reproduction should encourage future research on the behavioral, physiological and life history features of adaptability of these lizards as a result of co-evolution with their gastric nematodes.

Future research into the role of helminths into their hosts' physiology—especially for reptiles as hosts—is needed across many fields of study. Research on the pathology of other common reptilian parasites (*e.g.,* hepatozoon and filarial infestations) and ectoparasites are needed. Helminths (*e.g.,* nematodes and cestodes) acting as internal gastrointestinal competitors seem especially worthy of further scrutiny. The effects of nematodes on reptiles may be more nuanced than the ubiquitous negative effects in mammals, so studies into parasitism on ectotherms and invertebrates would provide much-needed detail into parasitism's place in the current understanding of tropic energy webs across multiple phylogenies. For example, a multi-year study comparing the severity of nematode infestation in years of high vs. low primary productivity and lizard reproductive output would be an excellent next step to enhance the *in situ* understanding of this system. Furthermore, a manipulative study utilizing anti-helminthic medications before or during the reproductive season would deepen the understanding of *S. phrynosoma* pathology. I hope that the breadth of this study will underscore the need for research relating parasitism with not just energetics and foraging, but also with endocrinology, immunology and epigenetics, thus connecting parasite load to the overall network of individual and population fitness across generations.

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APPENDIX

Results Statistics

Table 2: ANOVA results of differences in nematode load, snout-vent length and each response variable across sex, significant values listed in bold ($p<0.05$) * =multiple years of data used.

Independent Variable	**Response Variable**	**n**	**Df** **(sex, residual)**	**F-Stat**	**p-value**	**Eta^2**
Females *Males*	*Nematode Load*	20 19	1, 38	0.91	0.347	0.023
Females *Males*	***SVL****	98 93	1, 189	60.71	**4.27x10^{-13}**	**0.243**
Females *Males*	*Foraging Distance*	9 10	1, 17	0.08	0.778	0.004
Females *Males*	*Number of ants*	12 14	1, 24	1.035	0.319	0.415
Females *Males*	***Fecal Length***	16 12	1, 26	5.74	**0.024**	**0.146**
Females *Males*	***Fecal Mass***	16 12	1, 23	4.35	**0.048**	**0.159**
Females *Males*	***Percent POCA****	99 37	1, 83	31.23	**2.83x10^{-7}**	**0.273**
Females *Males*	*Home Range Size**	26 26	1, 50	3.05	0.086	0.057
Females *Males*	*Velocity*	12 10	1, 20	1.86	0.187	0.085
Females *Males*	*Endurance*	12 10	1, 20	0.054	0.817	0.002
Females *Males*	***BCI****	91 93	1, 182	22.67	**3.9x10^{-6}**	**0.111**
Females *Males*	*Mites*	20 19	1, 38	0.352	0.557	0.009
Females *Males*	*Hematocrit*	7 9	1, 14	0.124	0.730	0.008
Females *Males*	*Blood Immune Response*	12 15	1, 25	3.84	0.061	0.133

Table 3: Type II OLS Regression results, significant values and comparisons ($p<0.05$) listed in bold. Where there was a significant difference between the sexes and thus data were separated out, sample size and regression stats will be listed separately by sex, where data were combined, sample size is combined.

Independent Variable	Response Variable	Sex	n	r^2	p-value	Regression Equation
SVL	***Nematode Load***	*Females*	20	0.025	0.249	$load = 0.63(SVL) - 36$
		Males	19	0.273	**0.013**	$load = 0.58(SVL) - 20$
SVL	***Forage Distance***	-	19	0.191	**0.031**	$foraging = 0.64(SVL) - 30$
SVL	*Number of ants*	-	26	0.016	0.272	$number\ ants = 0.72(SVL) + 87$
SVL	***Fecal Length***	-	28	0.239	**0.007**	$fecal\ length = 0.23(SVL) + 2$
SVL	***Fecal Mass***	-	28	0.215	**0.007**	$fecal\ mass = 0.04(SVL) - 0.1$
*SVL**	***Percent POCA****	***Females***	62	0.171	**>0.001**	$percent\ POCA = 1.17(SVL) - 38$
		Males	64	0.0005	0.4301	$percent\ POCA = 0.05(SVL) + 32$
*SVL**	*Home Range Size**	-	51	0.051	0.055	$home\ range\ = 25(SVL) + 597$
SVL	*Velocity*	-	22	0.012	0.315	$velocity = 0.72(SVL) + 87$
SVL	***Endurance***	-	22	0.176	0.057	$endurance = 0.10(SVL) + 5$
SVL*	***BCI****	***Females***	91	0.689	**>0.001**	$BCI = 0.63(SVL) - 36$
		Males	93	0.637	**>0.001**	$BCI = 0.55(SVL) - 19$
SVL	*Mites*	-	38	0.067	0.057	$number\ mites = 0.72(SVL) - 37$
SVL	*Hematocrit*	-	14	0.026	0.288	$hematocrit = -0.11(SVL) + 37$
SVL	*Immune Response*	-	25	0.075	0.092	$blood\ imm.resp. = -0.77(SVL) + 106$
Nematode Load	***Forage Distance***	-	19	0.238	**0.017**	$foraging = 3.6(load) + 7$
Nematode Load	*Number of ants*	-	26	0.080	0.120	$number\ ants = 0.89(load) + 138$
Nematode Load	*Fecal Length*	-	28	0.057	0.109	$fecal\ length = -0.74(load) + 21$
Nematode Load	*Fecal Mass*	-	25	0.086	0.076	$fecal\ mass = 0.02(load) + 0.14$
Nematode Load	*Percent POCA*	*Females*	12	0.184	0.081	$percent\ POCA = -9.3(load) + 71$
		Males	12	0.086	0.176	$percent\ POCA = -3.5(load) + 35$
Nematode Load	*Home Range Size*	-	18	0.156	0.052	$home\ range\ = 121(load) + 447$
Nematode Load	*Velocity*	-	22	0.017	0.277	$velocity = -0.02(load) + 0.62$
Nematode Load	*Endurance*	-	22	0.991	0.076	$endurance = -2.8(load) + 20$
Nematode Load	***BCI***	***Females***	16	0.165	**0.049**	$BCI = 1.06(load) + 23$
		Males	18	0.448	**0.001**	$BCI = 2.73(load) + 16$
Nematode Load	***Mites***	-	40	0.083	**0.035**	$number\ mites = 4.76(load) + 4$
Nematode Load	*Hematocrit*	-	16	0.140	0.331	$hematocrit = 0.62(SVL) + 27$
Nematode Load	*Immune Response*	-	27	0.023	0.221	$blood\ imm.resp. = -2.42(SVL) + 52$

Table 4: Results of ANCOVA to separate out effect of SVL covariate. If analysis was separated out due to significant differences between the sexes, stats will be listed separately

Response Variable	**Sex**	**n**	**df** (load, SVL, residuals)	**p-value (load)**	**p-value (SVL)**	**Trend description**
Foraging Distance	-	19	2, 1, 15	**0.057**	0.632	Positive with greater loads
Number of ants	-	26	2, 1, 21	0.932	0.498	-
Fecal Length	***Females***	16	2, 1, 12	0.139	**0.051**	Positive with greater loads for females
	Males	14	2, 1, 7	0.828	0.565	
Fecal Mass	*Females*	12	2, 1, 9	0.565	0.702	-
	Males	13	2, 1, 8	0.842	0.154	
Percent POCA	***Females***	12	1, 1, 8	**0.025**	0.113	Positive with greater loads for females
	Males	14	2, 1, 9	0.452	0.866	
Home Range Size	-	18	2, 1, 12	0.2097	0.599	-
Velocity	-	22	2, 1, 18	0.603	0.660	-
Endurance	-	22	2, 1, 18	**0.035**	0.448	Negative with greater loads
BCI	***Females***	16	2, 1, 12	0.082	**0.0006**	Positive with greater SVL
	Males	18	2, 1, 14	0.163	**0.009**	
Mites	-	40	2, 1, 34	0.312	0.391	-
Hematocrit	-	16	2, 1, 10	0.896	0.509	-
Blood Immune Response	-	27	2, 1, 21	0.056	0.229	negative with increasing loads (marginally insignificant)

Table 5: Results of tests to satisfy assumptions of normality (Shapiro-Wilkes test) and homogeneity of variance (Levene's test). Comparisons are deemed normal or homogenous at p>0.05.

Tested Variable	**Sex**	**Shapiro Wilkes p-value**	**Normal?**	**Levene's p-value**	**Variance Homogenous?**
Nematode Load	*Females*	0.231	Yes	0.387	Yes
	Males	0.420	Yes		
SVL	*Females*	0.231	Yes	0.050	Yes~
	Males	0.420	Yes		
Foraging Distance	*Females*	0.023	No	0.431	Yes
	Males	0.118	Yes		
Number of ants	*Females*	0.151	Yes	0.525	Yes
	Males	0.312	Yes		
Fecal Length	*Females*	0.429	Yes	0.997	Yes
	Males	0.139	Yes		
Fecal Mass	*Females*	0.185	Yes	0.157	Yes
	Males	0.788	Yes		
Percent POCA	*Females*	0.313	Yes	0.525	Yes
	Males	0.151	Yes		
# Nematodes	*Females*	0.303	Yes	0.916	Yes
	Males	0.008	No		
Home Range Size	*Females*	0.873	Yes	0.522	Yes
	Males	0.035	No		
Velocity	*Females*	0.105	Yes	0.2236	Yes
	Males	0.253	Yes		
Endurance	*Females*	0.670	Yes	0.7398	Yes
	Males	0.057	No		
BCI (simple)	*Females*	0.882	Yes	0.020	No
	Males	0.652	Yes		
BCI (scaled)	*Females*	0.992	Yes	0.5735	Yes
	Males	0.093	Yes		
Mites	*Females*	0.002	No	0.9122	Yes
	Males	0.00045	No		
Hematocrit	*Females*	0.0018	No	0.6659	Yes
	Males	0.1126	Yes		
Blood Immune Response	*Females*	0.0004	No	0.3545	Yes
	*Female***	0.104**	Yes**		
*** Outlier removed*	*Male*	0.391	Yes	0.5849**	Yes**

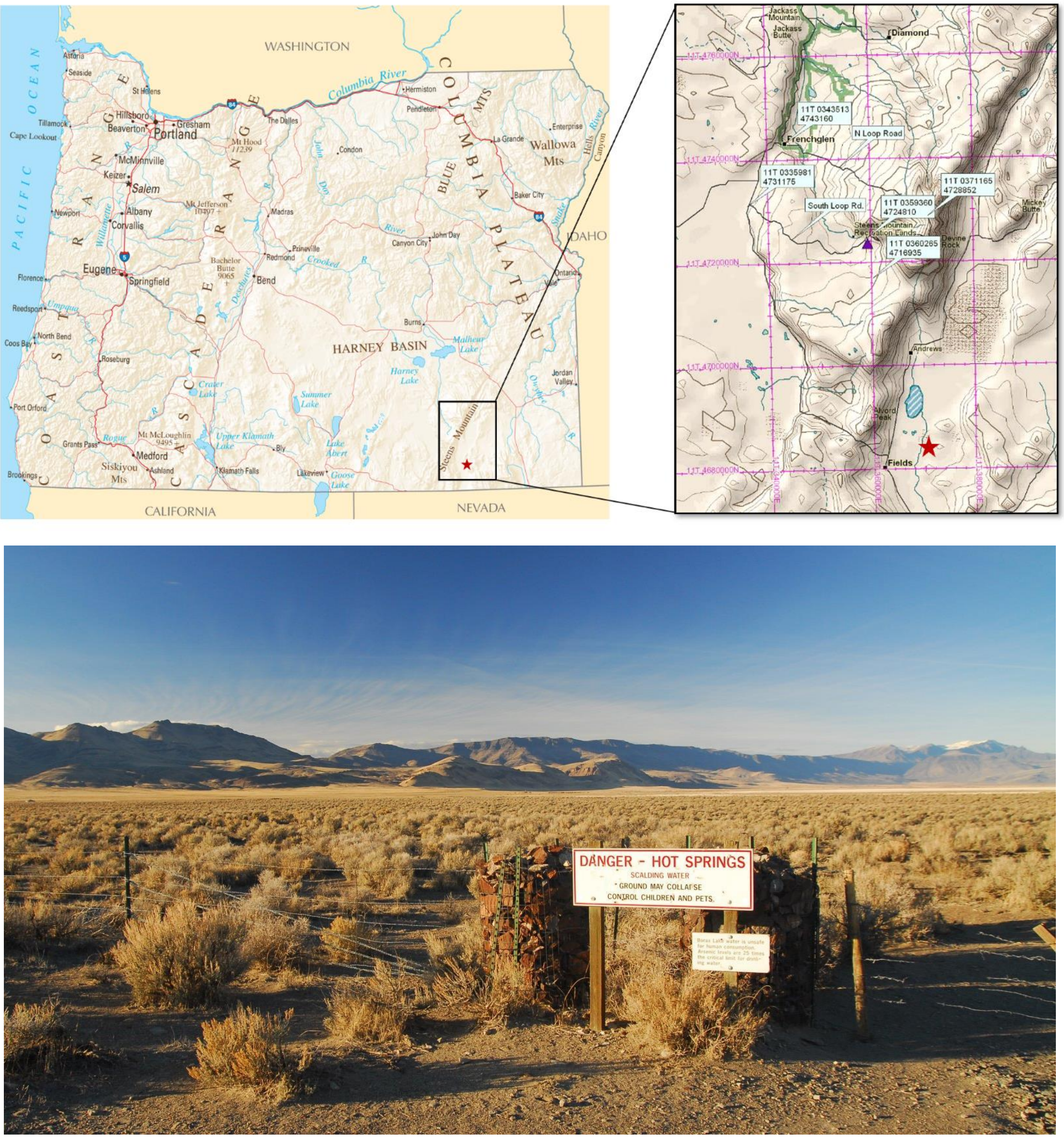
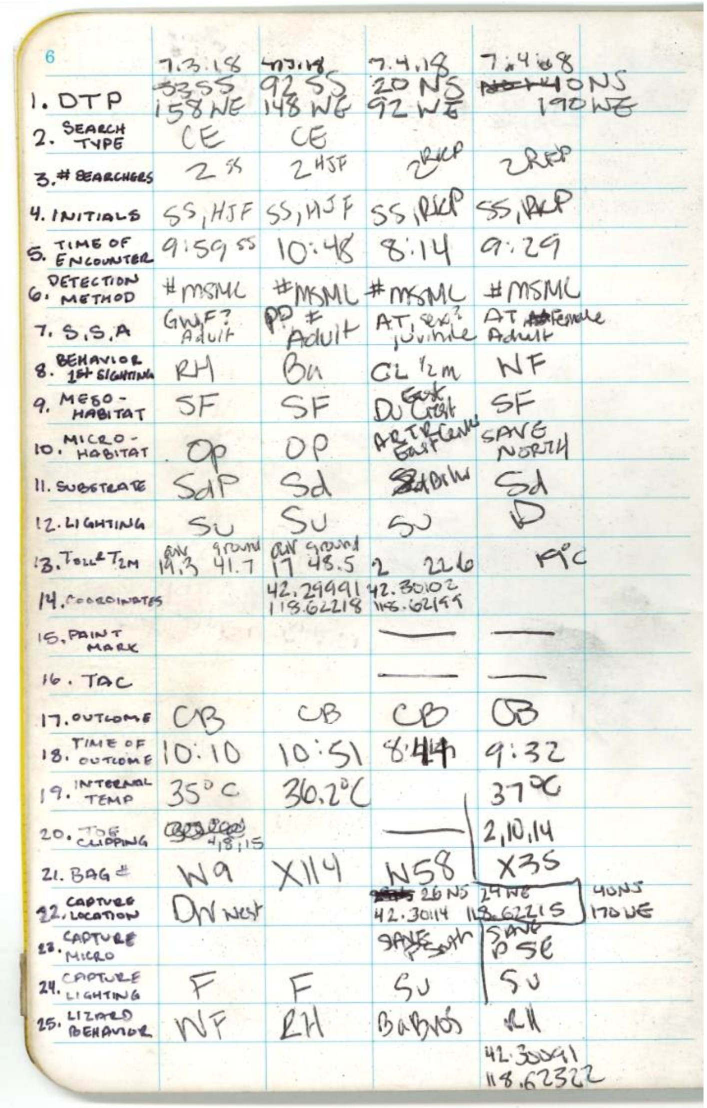
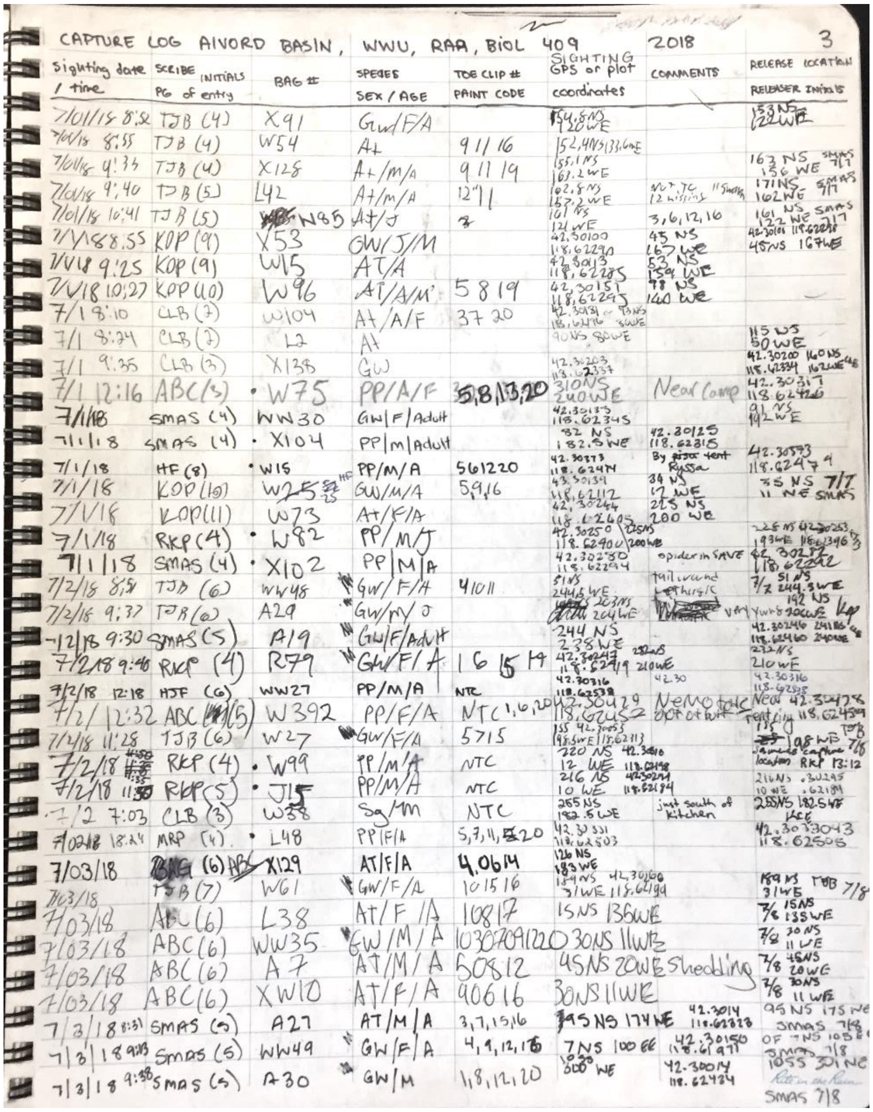


Figure 36: Map and photograph of the field site located in the Alvord Desert, the northern extent of the Great Basin Desert, in Harney county, OR, at approximately 42.303043, latitude, -118.624063 longitude.



6					
	7.3.18	7.3.18	7.4.18	7.4.18	
1. DTP	33SS 158NE	92SS 148WE	20NS 92WE	~~[illegible]~~ 40NS 190WE	
2. SEARCH TYPE	CE	CE			
3. # SEARCHERS	2 SS	2 HJF	2 RKP	2 RKP	
4. INITIALS	SS, HJF	SS, HJF	SS, RKP	SS, RKP	
5. TIME OF ENCOUNTER	9:59 55	10:48	8:14	9:29	
6. DETECTION METHOD	#MSML	#MSML	#MSML	#MSML	
7. S.S.A	GWF? Adult	PD # Adult	AT, sex? juvinile	AT ~~[illegible]~~ Female Adult	
8. BEHAVIOR 1st SIGHTING	RH	Bu	CL ½m	NF	
9. MESO-HABITAT	SF	SF	DU East Creek	SF	
10. MICRO-HABITAT	Op	OP	ARTR Center East	SAVE NORTH	
11. SUBSTRATE	SdP	Sd	~~Sd~~ SdBrW	Sd	
12. LIGHTING	SU	SU	SU	D	
13. T_{soil} & T_{2M}	air 19.3 ground 41.7	air 17 ground 48.5	2 22.6	19°C	
14. COORDINATES		42.29991 118.62218	42.30102 118.62199		
15. PAINT MARK			—	—	
16. TAC			—	—	
17. OUTCOME	CB	CB	CB	CB	
18. TIME OF OUTCOME	10:10	10:51	8:44	9:32	
19. INTERNAL TEMP	35°C	36.2°C		37°C	
20. TOE CLIPPING	~~[illegible]~~ 4,18,15		—	2,10,14	
21. BAG #	W9	X114	W58	X35	
22. CAPTURE LOCATION	DW west		~~[illegible]~~ 26NS 24WE 42.30114 118.62215		40NS 170WE
23. CAPTURE MICRO			SAVE P South	SAVE P SE	
24. CAPTURE LIGHTING	F	F	SU	SU	
25. LIZARD BEHAVIOR	WF	RH	BaBVOS	RH	
				42.30091 118.62322	

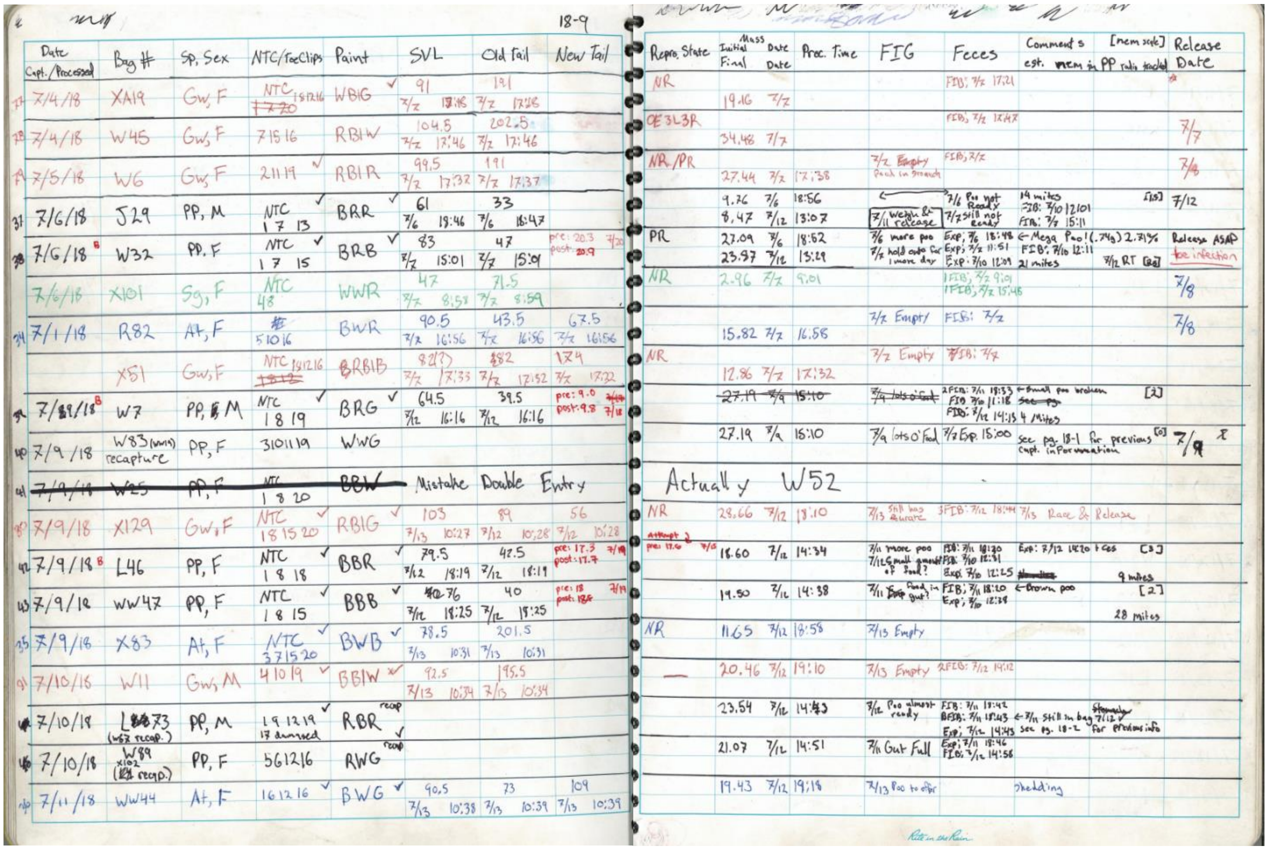
Figure 37: Scanned section of a 2018 student’s field notebook, indicating scribing procedures for lizard capture. Each column represents an individual lizard capture, and reach row represents an itemized capture detail, these being: date and plot, search type, # of searchers, initials of scribe and searchers, event time, detection method, species-sex-age, behavior at detection, mesohabitat, microhabitat, substratum, lighting on lizard at sighting, temperature of substate and 2M above, location of capture, paint mark status, transmitter condition (if applicable), outcome, time of outcome, If captured: body temperature, toe clip status, bag#, capture location, capture microhabitat, capture lighting, lizard behavior, comments.



CAPTURE LOG AIVORD BASIN, WWU, RAA, BIOL 409 2018 3

Sighting date / time	SCRIBE INITIALS PG of entry	BAG #	SPECIES SEX / AGE	TOE CLIP # PAINT CODE	SIGHTING GPS or plot coordinates	COMMENTS	RELEASE LOCATION RELEASER INITIALS
7/01/18 8:52	TJB (4)	X91	Gw/F/A		154.8NS 120WE		153NS 122WE
7/01/18 8:55	TJB (4)	W54	At	9 11 16	152.4NS 133.6WE		
7/01/18 9:33	TJB (4)	X128	At/m/A	9 11 19	155.1NS 163.2WE		163 NS 156 WE SMAS 7/7
7/01/18 9:40	TJB (5)	L42	At/m/A	12 11	162.8NS 157.2WE	NOT TC 12 missing	171NS 162WE SMAS 7/7
7/01/18 10:41	TJB (5)	W85	At/J		161 NS 121 WE	3,6,12,16	161 NS 122 WE SMAS 7/7
7/1/18 8:55	KDP (9)	X53	GW/J/M		42.30100 118.62290	45 NS 167 WE	42.30106 118.62298 45NS 167WE
7/1/18 9:25	KOP (9)	W15	AT/A		42.30113 118.62285	53 NS 159 WE	
7/1/18 10:27	KOP (10)	W96	AT/A/M	5 8 19	42.30151 118.62295	98 NS 140 WE	
7/1 8:10	CLB (7)	W104	At/A/F	37 20	42.30181 or 93NS 118.62196 80WE		
7/1 8:24	CLB (7)	L2	At		90NS 80WE		115 NS 50 WE
7/1 9:35	CLB (3)	X138	GW		42.30203 118.62337		42.30200 160NS 118.62334 162WE
7/1 12:16	ABC (3)	• W75	PP/A/F	5,8,13,20	310NS 240WE	Near Camp	42.30317 118.62426
7/1/18	SMAS (4)	WW30	GW/F/Adult		42.30135 118.62345		91 NS 192 WE
7/1/18	SMAS (4)	• X104	PP/m/Adult		82 NS 182.5 WE	42.30125 118.62315	
7/1/18	HF (8)	• W15	PP/M/A	561220	42.30373 118.62474	By ~~pisa~~ tent Russa	42.30593 118.62479
7/1/18	KOP (10)	W25 HF	GW/M/A	5,9,16	43.30134 118.62112	34 NS 17 WE	35 NS 7/7 11 WE SMAS
7/1/18	KOP (11)	W73	At/F/A		42.30244 118.62405	225 NS 200 WE	
7/1/18	RKP (4)	• W82	PP/M/J		42.30250 225NS 118.62400 200WE		225 NS 42.30253 193WE 118.62396 7/3
7/1/18	SMAS (4)	• X102	PP/M/A		42.30280 118.62294	spider in SAVE	42.30282 118.62292
7/2/18 8:51	TJB (6)	WW48	GW/F/A	41011	51NS 244.5WE	tail wound lethargic	7/2 51 NS 244.3WE
7/2/18 9:37	TJB (6)	A29	GW/M/J		203NS 204WE	very young	192 NS 206WE KP
7/2/18 9:30	SMAS (5)	A19	GW/F/Adult		244 NS 238 WE		42.30246 241NS 118.62460 240WE
7/2/18 9:46	RKP (4)	R79	GW/F/A	1 6 15 14	42.30247 232NS 118.62419 210WE		232NS 210WE
7/2/18 12:18	HJF (6)	WW27	PP/M/A	NTC	42.30316 118.62538	42.30	42.30316 118.62538
7/2/ 12:32	ABC (5)	W392	PP/F/A	NTC 1,6,20	42.30429 118.62452	Nemo tale out of tent	Near 42.30428 tenting 118.62459
7/2/18 11:28	TJB (6)	W27	GW/F/A	5715	135 42.30053 198.5WE 118.62313		135 198WE TJB 7/8
7/2/18 11:35	RKP (4)	• W99	PP/M/A	NTC	220 NS 42.3010 12 WE 118.62198		same as capture location RKP 13:12
7/2/18 11:30	RKP (5)	• J15	PP/M/A	NTC	216 NS 42.30211 10 WE 118.62184		216NS .30295 10WE .62184
7/2 7:03	CLB (3)	W38	Sg/M	NTC	255NS 182.5WE	just south of kitchen	255NS 182.5WE KCE
7/02/18 18:24	MRP (4)	• L48	PP/F/A	5,7,11,20	42.30331 118.62503		42.3033043 118.62505
7/03/18	~~BNG~~ (6) ~~ABC~~	X129	AT/F/A	4,06 14	126 NS 183 WE		
7/03/18	TJB (7)	W61	GW/F/A	10 15 16	159NS 42.30160 31WE 118.62199		159 NS TJB 7/8 31WE
7/03/18	ABC (6)	L38	At/F/A	10817	15NS 136WE		7/8 15NS 135WE
7/03/18	ABC (6)	WW35	GW/M/A	10307091220	30NS 11WE		7/8 30NS 11 WE
7/03/18	ABC (6)	A7	AT/M/A	50812	45NS 20WE	Shedding	7/8 45NS 20WE
7/03/18	ABC (6)	XW10	AT/F/A	90616	30NS 11WE		7/8 30NS 11 WE
7/3/18 8:31	SMAS (5)	A27	AT/M/A	3,7,15,16	95NS 174WE	42.3014 118.62323	95 NS 175 WE SMAS 7/8
7/3/18 9:13	SMAS (5)	WW49	GW/F/A	4,9,12,16	7NS 100WE	42.30150 118.61971	OF 7NS 105E SMAS 7/8
7/3/18 9:38	SMAS (5)	A30	GW/M	1,8,12,20	1055 300 WE	42.30014 118.62434	1055 301WE Rite in the Rain SMAS 7/8

Figure 38: Scanned section of the 2018 Lizard Capture Log, which records all lizard captures and releases. Column left to right are: Sighting date/time, Scribe Initials, Species Sex/Age, Toe Clip # and Paint Code, Comments, Release Location and Releaser Initials, each row is an individual lizard capture.



18-9

	Date Capt./Processed	Bag #	Sp, Sex	NTC/ToeClips	Paint	SVL	Old Tail	New Tail	Repro. State	Mass Initial Final	Date Date	Proc. Time	FIG	Feces	Comments est. nem in PP	[nem scale] radio tracked	Release Date
77	7/4/18	XA19	Gw, F	NTC 18 12 16 ~~1 7 20~~	WBIG ✓	91 7/7 17:48	191 7/7 17:48		NR	19.16	7/7			FIB; 7/7 17:21			☆
78	7/4/18	W45	Gw, F	7 15 16	RBIW	104.5 7/7 17:46	202.5 7/7 17:46		OE3L3R	34.48	7/7			FIB; 7/7 17:47			7/7
79	7/5/18	W6	Gw, F	2 11 19 ✓	RBIR	99.5 7/7 17:32	191 7/7 17:37		NR/PR	27.44	7/7	17:38	7/7 Empty Pooh in stomach	FIB; 7/7			7/8
37	7/6/18	J29	PP, M	NTC ✓ 1 7 13	BRR ✓	61 7/6 18:46	33 7/6 18:47			9.76 8.47	7/6 7/12	18:56 13:07	⟷ 7/11 weigh & release	7/6 Poo not Ready 7/7 still not ready	14 mites FIB: 7/10 12:01 FIB: 7/7 15:11	[15]	7/12
38	7/6/18 B	W32	PP, F	NTC ✓ 1 7 15	BRB ✓	83 7/7 15:01	47 7/7 15:09	pre: 20.3 7/20 post: 20.9	PR	27.09 23.97	7/6 7/12	18:52 13:29	7/6 more poo 7/7 hold onto for 1 more day	Exp; 7/6 18:48 Exp; 7/7 11:51 Exp: 7/10 12:09	← Mega Poo! (.74g) 2.71% FIB: 7/10 12:11 21 mites	7/12 RT [80]	Release ASAP toe infection
	7/6/18	X101	Sg, F	NTC 48	WWR	47 7/7 8:58	71.5 7/7 8:59		NR	2.96	7/7	9:01		1FIB; 7/7 9:01 1FIB; 7/7 15:48			7/8
34	7/1/18	R82	At, F	5 10 16	BWR	90.5 7/7 16:56	43.5 7/7 16:56	67.5 7/7 16:56		15.82	7/7	16:58	7/7 Empty	FIB: 7/7			7/8
		X51	Gw, F	NTC 18 12 16 ~~18 12~~	BRBIB	82(?) 7/7 17:33	82 7/7 17:52	174 7/7 17:32	NR	12.86	7/7	17:32	7/7 Empty	FIB: 7/7			
39	7/9/18 B	W7	PP, M	NTC ✓ 1 8 19	BRG ✓	64.5 7/12 16:16	39.5 7/12 16:16	pre: 9.0 post: 9.8 7/18		~~27.19~~	~~7/9~~	~~15:10~~	~~7/9 lots o' food~~	2FIB: 7/11 18:33 FIB 7/10 11:18 FIB: 7/12 14:13	← Small poo broken ~~see pg.~~ 4 Mites	[2]	
40	7/9/18	W83 (WW19) recapture	PP, F	3 10 11 19	WWG					27.19	7/9	15:10	7/9 lots o' Food	7/7 Exp. 15:00	see pg. 18-1 for previous capt. information	[0]	7/9 ✗
41	~~7/9/18~~	~~W25~~	~~PP, F~~	~~NTC~~ 1 8 20	~~BBW~~	Mistake	Double	Entry	Actually W52								
80	7/9/18	X129	Gw, F	NTC ✓ 18 15 20	RBIG ✓	103 7/13 10:27	89 7/12 10:28	56 7/12 10:28	NR	28.66	7/12	18:10	7/13 Still has urate	3FIB: 7/12 18:44	7/13 Race & Release		
42	7/9/18 B	L46	PP, F	NTC ✓ 1 8 18	BBR ✓	79.5 7/12 18:19	42.5 7/12 18:19	pre: 17.3 7/19 post: 17.7	Attempt 2 pre: 17.6 7/5	18.60	7/12	14:34	7/11 more poo 7/12 Small amount of food?	FIB: 7/11 18:20 FIB: 7/10 12:31 Exp: 7/10 12:25	Exp: 7/12 14:20 + Ces	[3] 9 mites	
43	7/9/18	WW47	PP, F	NTC ✓ 1 8 15	BBB ✓	~~40~~ 76 7/12 18:25	40 7/12 18:25	pre: 18 7/19 post: 18.5		19.50	7/12	14:38	7/11 ~~Exp~~ food in gut?	FIB; 7/11 18:20 Exp; 7/10 12:38	← Brown poo	[2] 28 mites	
35	7/9/18	X83	At, F	NTC ✓ 3 7 15 20	BWB ✓	78.5 7/13 10:31	201.5 7/13 10:31		NR	11.65	7/12	18:58	7/13 Empty				
81	7/10/18	W11	Gw, M	4 10 19 ✓	BBIW ✓	92.5 7/13 10:34	195.5 7/13 10:34		—	20.46	7/12	19:10	7/13 Empty	2FIB: 7/12 19:12			
44	7/10/18	L73 (W67 recap.)	PP, M	1 9 12 19 ✓ 17 damaged	RBR recap ✓					23.54	7/12	14:43	7/12 Poo almost ready	FIB: 7/11 18:42 BFIB: 7/11 18:43 Exp; 7/12 14:43	← 7/11 still in bag see pg. 18-2	~~Stomach~~ 7/12 ✓ for previous info	
46	7/10/18	W89 X102 (recap.)	PP, F	5 6 12 16	RWG recap					21.07	7/12	14:51	7/11 Gut Full	Exp; 7/11 18:46 FIB; 7/12 14:56			
	7/11/18	WW44	At, F	16 12 16 ✓	BWG ✓	90.5 7/13 10:38	73 7/13 10:39	109 7/13 10:39		19.43	7/12	19:18	7/13 Poo to offer		shedding		

Rite in the Rain

Figure 39: Scanned section of 2018 Lizard body data log, where all lizard processing data, morphometrics, fecal collection, reproductive state and parasite information is recorded. Each row is an individual lizard, each column is a detail of lizard processing and morphometrics. Columns from left to right are: date, bag #, species and sex, toe clip #, paint code, SVL, old tail length, new tail length, female repro-state, masses and mass date, processing Time, feces in bag (FIB), other feces, comments, estimated nematodes and number of mites, release date.

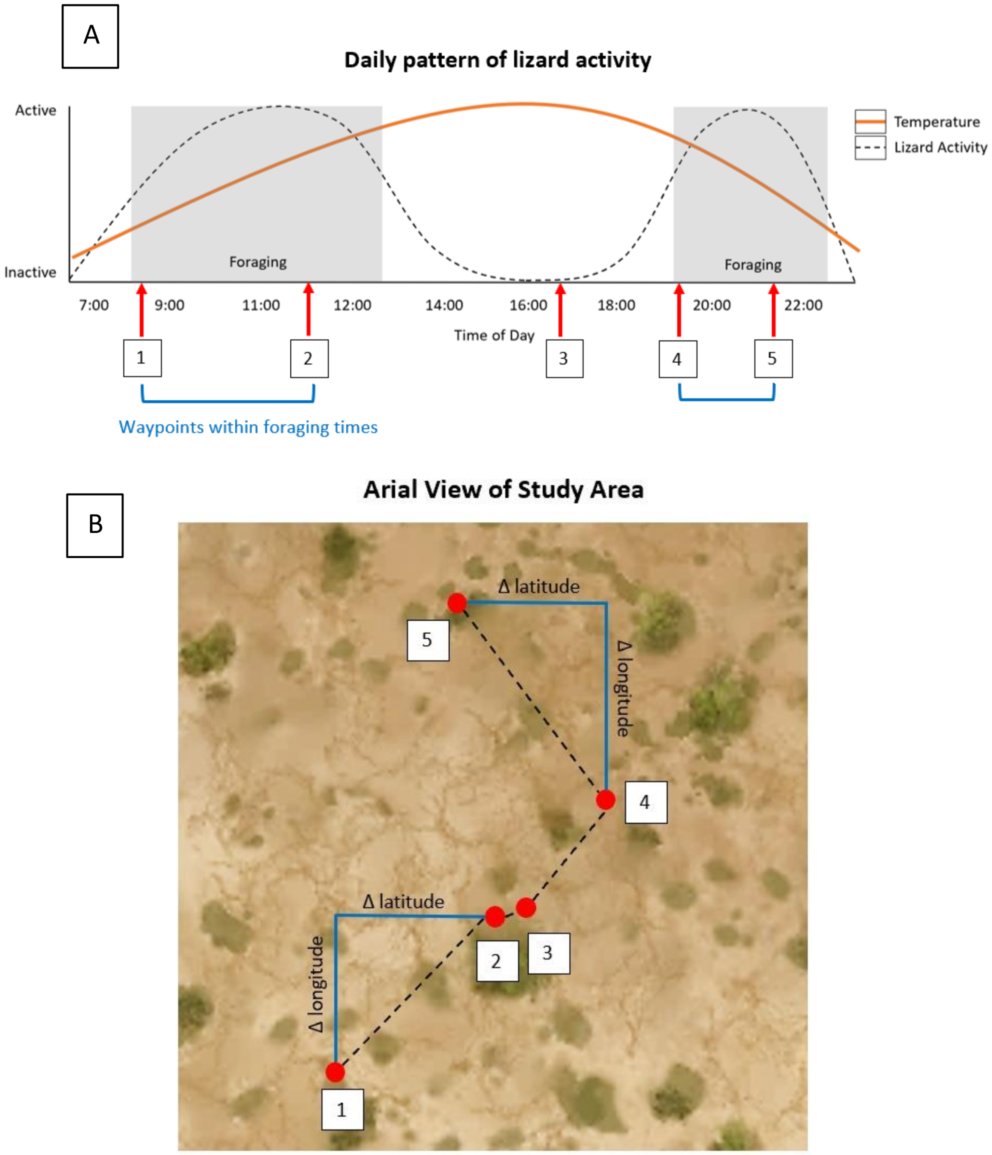


Figure 40: Part **A)** The pattern of horned lizard activity (dotted line) with foraging periods shaded in gray. Red lines indicate sighting waypoints, and blue brackets indicate waypoints appropriate to be used in calculating foraging distance (1-2), (4-5). Part **B)** represents the same waypoints as they exist spatially on the landscape. The change in latitude and longitude between sets of waypoints, represented in blue lines was used to calculate the distance between waypoints (1-2) and (4-5).

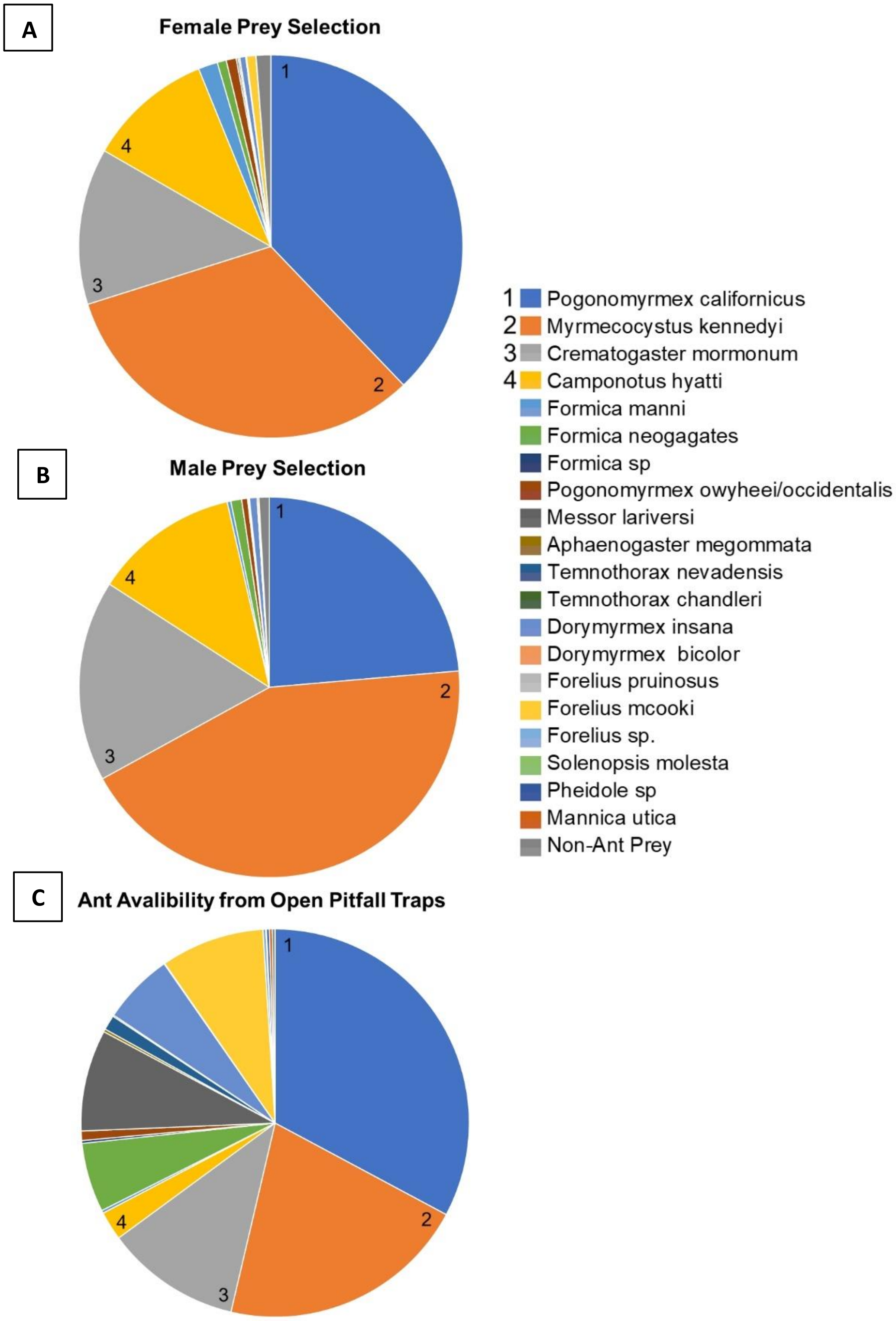


Figure 41: Comparisons of proportion of ant species found in fecal pellets (n=32) to those found in open pitfall traps (n=21 pairs). **A)** Compares female prey choice to environmental ant availability, **B)** Compares male prey choice to environmental ant availability and **C)** compares prey choice of male vs female horned lizards. Legend goes clockwise on pie chart, most common four species numbered for ease of comparison.