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Host and Seasonal Effects on the Infection Dynamics of Skrjabinoptera Phrynosoma (Ortlepp) Schulz, 1927, a Parasitic Nematode of Horned Lizards

Kathryn Claire Hilsinger

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HOST AND SEASONAL EFFECTS ON THE INFECTION DYNAMICS OF
SKRJABINOPTERA PHRYNOSOMA (ORTLEPP) SCHULZ, 1927, A PARASITIC
NEMATODE OF HORNED LIZARDS

by

KATHRYN CLAIRE HILSINGER

Under the direction of Dana Nayduch

ABSTRACT

Skrjabinoptera phrynosoma (Ortlepp) Schulz, 1927 is a common parasitic nematode of horned lizards. The life cycle of *S. phrynosoma* was described by Lee in 1957, but has received little attention since. The present study addressed effect of season as well as host characteristics on the infection dynamics in lizard hosts. In the Alvord Basin in southeastern Oregon, *S. phrynosoma* were collected from *Phrynosoma platyrhinos* Gerard 1852 horned lizards via stomach flushes, cloaca flushes and fecal pellet collections. Parasite load variables (number of nematodes per host, length of those nematodes, and total worm burden (ΣL)) were analyzed within three collection periods during the active season of 2008. Number and length of nematodes of different sex categories also was analyzed within collection period and across season. The relationship between parasite variables and host characteristics (sex and SVL) were analyzed. *Pogonomyrmex* spp. harvester ants were collected and dissected to determine the prevalence of infection in this intermediate host. The number of non-gravid female nematodes as well as the number of juvenile nematodes in lizards' stomachs decreased significantly between the early and late collection periods. While the number of male nematodes in lizards' stomachs did not change across season, the length of male

nematodes increased significantly between early, middle and late collection periods. During the early collection period, host SVL was positively correlated with non-gravid female nematode length and juvenile nematode length. Also, in late season, there was a negative relationship between lizard SVL and number of gravid female nematodes. Nematodes were retrieved from cloacal sampling mostly during the middle collection period, and were exclusively gravid female nematodes. Prevalence in the ant intermediate host was extremely low. As the population of male nematodes in lizards' stomachs remains stable, it is proposed that any newly-establishing nematodes (juveniles) develop into non-gravid females and then, after mating, develop into gravid female nematodes. It is also proposed that in larger lizards, newly-establishing nematodes (juveniles) can develop into females, can mate, and can exit the lizard faster because of more space and resources in the larger stomachs. The changing parasite load of *S. phrynosoma* in *P. platyrhinos* across the active season is most likely driven by the timing of the unique life cycle of this parasite.

INDEX WORDS: *Skrjabinoptera phrynosoma*, Parasitic nematode, Parasite life cycle, Parasite of horned lizard

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KATHRYN CLAIRE HILSINGER

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KATHRYN CLAIRE HILSINGER

Major Professor: Dana Nayduch
Committee: Lance McBrayer
Oscar Pung

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CHAPTER 1

SEASONAL EFFECTS ON THE INFECTION DYNAMICS OF *SKRJABINOPTERA*
PHRYNOSOMA (ORTLEPP) SCHULZ, 1927, A PARASITIC NEMATODE OF
HORNED LIZARDS

ABSTRACT

The parasitic nematode *Skrjabinoptera phrynosoma* (Ortlepp) Schulz, 1927 was collected from the desert horned lizard *Phrynosoma platyrhinos* Gerard, 1852 and the harvester ant *Pogonomyrmex* spp. to describe seasonal variation of parasite load in these hosts in the Alvord Basin of southeastern Oregon. Nematodes were collected from lizard stomach flushes, cloaca flushes and fecal pellets, and number of nematodes per host, length of those nematodes, and total worm burden were analyzed across active season. Number and length of nematode sex categories also were analyzed within collection period and across season. The number of non-gravid female nematodes as well as the number of juvenile nematodes in lizards' stomachs decreased significantly between early and late collection period. While the number of male nematodes in lizards' stomachs did not change across season, the length of male nematodes increased significantly between early, middle and late collection period. It is proposed that the population of male nematodes in lizards' stomachs remains stable, and any newly-establishing nematodes (juveniles) develop into non-gravid females and then, after mating, develop into gravid female nematodes and exit the lizard host to continue the remainder of the life cycle in the intermediate host. This is further supported by the peak of gravid female nematodes in cloacal collections during the middle season. One out of 6,000 dissected

Pogonomyrmex spp. ants was infected with a larval nematode. This ant was collected during the early collection period. The changing parasite load of *S. phrynosoma* in *P. platyrhinos* across the active season is most likely driven by the timing of the unique life cycle of this parasite.

INTRODUCTION

Seasonality of parasite infection rate may be influenced by fluctuations in the hosts' exposure to the infective stages (Cornell et al. 2008). Additionally, the availability and transmission of parasites is affected by environmental factors such as temperature and moisture (Stromberg 1997). For example, environmental factors influence the availability and transmission of monoxenous parasites mainly by affecting the survival of free-living forms (Stromberg 1997, Tubbs et. al 2004, Calero-Torralbo 2008). However, for parasites with heteroxenous life cycles, environmental factors influence host characteristics and life cycles which results in variation of parasite abundance within these hosts. For example, the occurrence of the malaria parasite, *Plasmodium falciparum*, in human hosts living in two climatic zones in Ethiopia fluctuated due to the effect of temperature and rainfall on the mosquito host's life cycle (Teklehaimanot et al. 2004).

When ingestion of an insect intermediate host is necessary for transmission of a heteroxenous parasite, this transmission is limited to the active period of the insect host. For example, infection with the nematode *Spinitectus carolini* in largemouth bass peaked in July, probably due to the abundance and utilization of the insect intermediate host as a food source by the fish during spring and summer (Ingham and Dronen 1982). Similarly,

infection of frog definitive hosts with the lung fluke *Haematoloechus coloradensis*, whose intermediate hosts include naiads of dragonflies and damselflies, also varies seasonally (Dronen 1978, Marin et al. 1997). Odonate naiads are a food source for frogs, and the presence of infected Odonate naiads in the environment from April to June results in an increase in infection of *H. coloradensis* in frogs during May, June and July.

Seasonal changes in host reproduction or activity (e.g. hibernation) also have been shown to affect parasite infection rates. Seasonal breeding and the resulting fluctuation in certain hormones such as testosterone may suppress immunity in organisms (Folstad and Karter 1992, Hosseini et al. 2004), and elevated testosterone levels have been linked to increases parasite load in several animals (Poulin 1996, Zuk and McKean 1996, Schalk and Forbes 1997). In contrast, hibernation results in an increased resistance to parasite infection (Kalabukhov 1958, Chute 1961, Kayser 1961), possibly due to changes in the host such as lowered body temperature and metabolism, which may slow the development of parasites (Kayser 1961). For example, after a period of induced hibernation, preexisting helminth infections in the 13-line ground squirrel were either eliminated or greatly decreased (Chute 1961, Cahill et al. 1967).

The present study addresses seasonal variation in infection of the nematode *Skrjabinoptera phrynosoma* (Spirurida: Physalopterinae) (Ortlepp) Schulz, 1927 in its hosts. The heteroxenous life cycle of this parasite was first described in the Texas horned lizard *Phrynosoma cornutum* (Lee 1957). Instead of passing eggs in the feces of the definitive host, as most physalopterines do, whole gravid females of *S. phrynosoma* exit with feces of the lizard definitive host. These females, containing egg packets that may be viable for up to a year, die on the desert floor and are subsequently collected by

foraging harvester ants, *Pogonomyrmex* spp. (Lee 1955). Upon return to the nest, the foraging ants feed the body of the nematode, along with the encapsulated eggs, to larval ants. As the infected larval ants go through metamorphosis, the larval *S. phrynosoma* molt within the ant and eventually reach the third larval stage, which is infective to the lizard definitive host. The life cycle is completed when the foraging adult ant, containing the infective third larval stage, is eaten by the *Phrynosoma* lizard. *Skrjabinoptera phrynosoma* is commonly recovered from the desert horned lizard *Phrynosoma platyrhinos* Gerard, 1852 (Grundmann 1959; Waitz 1961, Haukisalmi et al. 1996) in surveys, but the infection dynamics of this particular host/parasite system has not been studied.

Describing the population dynamics of *S. phrynosoma* within *P. platyrhinos* and *Pogonomyrmex* spp. across active season is integral to understanding this unique life cycle. *Phrynosoma platyrhinos* and *Pogonomyrmex* spp., two hosts found in the northern arid regions of North America, exhibit seasonality in that they are active only during the warmer months (approximately from May through September). *Phrynosoma platyrhinos* hibernate during the winter, and the breeding season occurs shortly after emergence from hibernation (Pianka and Parker 1975). *Phrynosoma platyrhinos* may experience seasonal reproductive stresses because both males and females expend a great deal of energy on reproduction. *Phrynosoma* spp. clutch weight makes up a greater percentage of female body weight compared to other lizard species, producing a greater number of offspring (Pianka and Parker 1975). Additionally, male *Phrynosoma* spp. also increase their home range during breeding season, presumably to intercept more females (Stark et al 2005), and hormones of this genus fluctuate across the active season (Wack et al. 2007). Given

the seasonality of the hosts of *S. phrynosoma* in this system, the following questions were addressed: 1.) What is the parasite load and sex distribution of *S. phrynosoma* in *P. platyrhinos* within collection period and across active season? 2.) What is the prevalence of *S. phrynosoma* in *Pogonomyrmex* spp.? To answer these questions, nematodes were recovered from lizards by stomach flushing, cloaca flushing, and fecal pellet collection over the course of one active season. Numbers and lengths nematode sex categories were compared within lizards and across the active season. Total worm burden in lizards was analyzed across season as well. *Pogonomyrmex* spp. ants were pit-fall trapped and dissected to retrieve larval nematodes.

METHODS

Study area

The Alvord Basin (42°18'N, 118°37'W), in Harney County of southeastern Oregon, is at the northern end of the Great Basin Desert. This portion of Oregon's high desert lies mostly 3800 – 4000 feet above sea level (Orr et al. 1992) and is surrounded by the Steens Mountain range. There is drastic seasonality, with temperatures ranging from an average low of -18°C in winter to an average high of 32°C in summer, with an average annual rainfall not exceeding 16.5 cm (Western Regional Climate Center [Updated 2007]). This area is home to several species of lizard, most commonly the long-nosed leopard lizard *Gambelia wislizenii*, the Great Basin whiptail *Aspidoscelis tigris*, and *Phrynosoma platyrhinos*. Regional vegetation consists mostly of sagebrush and other low shrubs. The 400 × 400 m plot used for this study was initially established in by 1998 Dr. Roger Anderson and his Western Washington University reptile ecology field course.

Lizard measurements

Data were collected three times throughout the active season of 2008: early (soon after lizards emerged from hibernation and were breeding; May 16 - June 2; n = 13), middle (during female lizard egg laying; June 25 – July 15; n = 15), and late season (post-reproduction; August 1 – 15; n = 14). The 400 × 400 m plot was flagged every 25 meters, and plot coordinates were marked on the flags. Lizards were caught throughout the day by hand on or surrounding the 400 × 400 m plot and held individually in cloth bags. These bags were kept in insulated coolers to maintain stable temperatures. Lizards were sexed, weighed (to the nearest 0.1 g), measured (snout-vent length in mm (SVL)), and marked for identification to avoid resampling during subsequent collection periods.

Nematode collection

To retrieve *S. phrynosoma* from the digestive tract, lizards were stomach-flushed and cloaca-flushed within 1-5 days of capture. Stomach flushing is discussed by many authors as a gentle and commonly used method for assessing diet in reptiles and anurans (Legler and Sullivan 1979, Pietruszka 1981; Cannon 2003; Graczyk et al 1996; Harr 2000; Solé et al. 2005), and was previously used by Griffiths et al. (1998) to determine nematode prevalence and intensity in frillneck lizards. In the present study, stomach flushing was used to evaluate parasite infection in the stomach as an alternative to euthanizing individuals. Necropsy of two lizards from the same site from prior years revealed this technique to be effective: stomach from 2005 contained 27 nematodes; stomach from 2006 contained 45 nematodes.

To retrieve nematodes, lizards were stomach flushed with ambient temperature standard Ringer's solution (NaCl, 0.66%; KCl, 0.015%; CaCl₂, 0.015%; NaHCO₃,

0.02%). A 5 mm diameter rubber canula, attached to a 10 ml syringe was gently inserted down the esophagus and into the stomach until slight resistance of the pyloric side of the stomach was felt (Legler and Sullivan 1979; Solé et al. 2005). The 10 ml of Ringer's solution was pumped into the stomach with enough force to push nematodes and food particles out through the mouth. This process was repeated until the entire food bolus was flushed from the stomach (usually 2-3 flushes). Flushed stomach contents were preserved in 75% glycerin alcohol in 20 mL vials.

Fecal pellets were expressed by gently palpating the lower gut and cloaca on the ventral side of the lizard. Naturally-passed fecal pellets were also collected from the cloth bags that were used to hold lizards while in captivity. Nematodes expelled with fecal pellets were preserved in glycerin alcohol as above.

Cloaca flushing also was used to assess parasite infection in the colon and cloaca, following the methods of Cannon (2003) and Mader (1996). This procedure was performed several hours after lizards' stomachs were flushed, to minimize stress. While the lizard was under manual restraint, a plastic pipette with a diameter of 4.5 mm was introduced into the cloaca and no more than 1 ml of Ringer's solution was introduced and aspirated at a time. The aspirate was deposited in a 20 ml vial and preserved in glycerin alcohol. This procedure was repeated three times for each lizard.

Nematode analysis

Nematodes from stomach and cloaca flushes and fecal pellets were counted, measured (mm) and sexed using a stereomicroscope. Sex of nematodes was determined by presence of wing-like caudal alae in males and a curling of the caudal end in females (Babero and Kay 1967). Females were determined to be gravid by the presence of visible

egg packets (Lee 1957). Nematodes that did not have apparent male or female characteristics were classified as juveniles. Nematode worm burden, which was defined as total length of all nematodes in a stomach flush ($\sum L$), was calculated. This seemed the best indication of total biomass, because nematode mass had not been measured in the field. The width/length ratio of all nematode sex categories was not significantly different.

Statistical analysis

The relationships between number of nematodes of each sex category (juvenile, non-gravid female, gravid female, pooled female (non-gravid and gravid) and male) within each collection period (early, middle and late) were analyzed. The number of nematodes of each sex category within each lizard was compared within collection period using two-way ANOVAs without replication on raw or log transformed data, and non-parametric Friedman's tests on data that could not be normalized. A posteriori paired *t*-tests were performed on parametric data, and Wilcoxon signed-ranks tests were performed on non-parametric data to compare pairs of nematode sex categories. When comparing juveniles, pooled females and males, a Bonferoni correction allowed for *P* values under 0.017 to be significant. When comparing juveniles, non-gravid females, gravid females and males, a Bonferoni correction allowed for *P* values under 0.008 to be significant.

The relationships between the length of nematodes of each sex category (juvenile, non-gravid female, gravid female, and male) within each collection period (early, middle and late) were analyzed. The preceding analyses (two-way ANOVA or Friedman's test and a posteriori) were used to analyze mean nematode length per lizard of each sex

category within collection period. Lizards whose stomach flushes did not yield nematodes of a particular sex category were removed from the analyses of nematode length.

To determine seasonal changes in parasite load variables, Kruskal-Wallis tests were run on nematode number (total and per sex category) per lizard stomach, nematode length (per sex category; irrespective of host), and worm burden ($\sum L$) per lizard stomach. A posteriori non-parametric analyses, Mann-Whitney U-test with Bonferoni correction (significant $p < 0.017$), were used to compare collection periods because data could not be normalized with transformations.

To describe nematodes that were in the lower portion of the gastrointestinal tract in *P. platyrhinos*, nematode data from fecal pellet collections and cloaca flushes were combined. So few nematodes were collected from these methods that a chi-square test was used only to compare number of lizards with cloacal nematodes (presence/absence) between collection periods.

All analyses were performed using the statistical program JMP (SAS Institute Inc. 2001), and figures were produced using Microsoft Excel (Microsoft Corporation 2003).

Ant collection

On the 400 × 400 m plot, pit-fall traps were placed near 35-38 nests of *Pogomyrmex* spp. harvester ants during each of the three collection periods. Plastic cups were placed in the ground flush with the ground's surface, each about 20-30 cm NE and SW of the nest opening. Ethylene glycol was placed in the traps to serve as a killing agent and a preservative. Traps were checked daily and left open for up to 7 days. Ants were dissected under a stereomicroscope to determine presence of third-stage larval

nematodes in the gasters (Lee 1957). Ants were then preserved in 70% EtOH and larval nematodes were preserved in glycerine alcohol.

RESULTS

A total of 42 lizards were collected during this study (early collection period, $n = 13$; middle collection period, $n = 15$; late collection period, $n = 14$). Stomach flushes yielded *S. phrynosoma* from all but one lizard. When the variables of parasite load were analyzed between male and female lizards, there was no effect of lizard sex on total number of nematodes, length of nematodes, or total worm burden in lizards' stomachs during any part of the season ($P > 0.12$ for all analyses); therefore, male and female lizards were grouped together for the remaining analyses.

Comparative analyses of nematode sex categories within collection periods

The number of nematodes per lizard stomach was analyzed within collection period (Table 1, Fig. 1). During the early collection period, there were more female nematodes (pooled non-gravid and gravid) in lizard stomachs than there were males ($t = 3.753$, $df = 12$, $P = 0.003$) and juveniles ($t = -6.124$, $df = 12$, $P < 0.001$), and more non-gravid female nematodes than males (Signed-rank = -38.0 , $df = 12$, $P = 0.006$), gravid females (Signed-rank = 45.5 , $df = 12$, $P < 0.0001$) and juveniles (Signed-rank = 39.0 , $df = 12$, $P < 0.0001$). (Pooled female data not shown in Figure 1: Mean number of female nematodes per lizard stomach during Early collection period: 13.8 (mm) ± 3.35 SE (range 2 – 36) ;Middle collection period: 11.3 (mm) ± 3.68 SE (range 0 – 47); Late collection period: 3.1 (mm) ± 0.66 (range 1 – 9) (Table1). During the middle collection period, there were more female nematodes (pooled) in lizard stomachs than males ($t = 2.735$, df

= 14, $P = 0.016$) and juveniles (Signed-rank = 40.5, $df = 14$, $P = 0.003$). During the late collection period, there were more females (pooled) (Signed-rank = 37.5, $df = 13$, $P = 0.007$) and males ($t = -3.107$, $df = 13$, $P = 0.002$) than there were juveniles. Also during the late collection period, there were more males than non-gravid females (Signed-rank = 37.0, $df = 13$, $P = 0.002$), gravid females (Signed-rank = 39.5, $df = 13$, $P = 0.004$) and juveniles (Signed-rank = 32.5, $df = 13$, $P = 0.008$).

The mean length of nematodes per lizard stomach was analyzed within collection period (Fig. 2). During the early collection period, the length of juveniles was less than that of non-gravid female (Signed-rank = 33.0, $df = 10$, $P < 0.001$) and male nematodes (Signed-rank = 27.5, $df = 9$, $P = 0.002$). The length of gravid females was greater than that of males ($t = 3.838$, $df = 5$, $P = 0.012$), although this trend was not significant. During the middle collection period, the length of non-gravid females was significantly greater than that of juveniles ($t = -4.737$, $df = 8$, $P = 0.002$). Also during the middle collection period, the length of gravid females was greater than that of non-gravid female nematodes ($t = 7.040$, $df = 8$, $P < 0.001$) male nematodes ($t = 11.451$, $df = 8$, $P < 0.001$) and juvenile nematodes ($t = -13.211$, $df = 8$, $P < 0.001$). Additionally, length of male nematodes was greater than that of juveniles ($t = -8.665$, $df = 8$, $P < 0.001$). During the late collection period, length of gravid female nematodes was significantly greater than that of males ($t = 6.387$, $df = 8$, $P = 0.0014$) and marginally greater than that of non-gravid females (Signed-rank = -10.5, $df = 5$, $P = 0.031$) and juveniles (Signed-rank = 5.0, $df = 3$, $P = 0.125$) but sample sizes for pairwise comparisons were low because some lizards did not contain all nematode sex categories. The length of juvenile nematodes

was significantly less than that of male (Signed-rank = 18, $df = 7$, $P = 0.008$) and non-gravid female nematodes (Signed-rank = 18.0, $df = 7$, $P = 0.008$).

Analyses of nematode number, length and total burden across active season

Mean nematode number per lizard stomach was compared across the three collection periods (Table 1). Mean number of total nematodes per lizard stomach during the early collection period was 23.0 ± 4.8 SE (range = 7-54), and mean number during the middle collection period was 18.9 ± 5.34 (range = 0-66). The mean nematode number per lizard during the late collection period was 8.7 ± 1.5 (range = 2-26), which resulted in a significant decrease in mean nematode number per lizard from early to late season (Mann-Whitney U-test: $U = 142.5$, $Z = 2.485$, early $n = 13$, late $n = 14$, $P = 0.013$). This pattern was driven by a decrease in the number of non-gravid females ($U = 12$, $Z = 3.891$, early $n = 13$, late $n = 14$, $P < 0.001$, Fig. 3), as well as a decrease in the number of juveniles ($U = 36$, $Z = 2.175$, early $n = 13$, late $n = 14$, $P = 0.007$).

The mean lengths of nematodes (irrespective of host) were compared between collection periods (Table 2, Fig. 4). The length of male nematodes was significantly greater during the middle collection period than the early collection period ($U = 3570$, $Z = -3.130$, early $n = 68$, middle $n = 81$, $P = 0.0017$) and greater during the late collection period than the middle collection period ($U = 3737$, $Z = 5.243$, middle $n = 61$, late $n = 81$, $P < 0.0001$, Figure 2b). The length of juveniles during the middle collection period was greater than that of the early collection period ($U = 1039$, $Z = 3.745$, early $n = 42$, middle $n = 33$, $P = 0.0002$), but the length of juveniles during the late collection period was significantly less than that of the middle collection period ($U = 437$, $Z = 2.801$, middle $n = 18$, late $n = 33$, $P = 0.0049$).

Finally, total worm burden per lizard was analyzed across the three collection periods (Table 3, Fig 5). Mean worm burden (ΣL) in lizard stomachs during the early collection period was 139.0 mm \pm 31.5 (range = 32 – 348 mm). Mean worm burden during the middle collection period was 142.2mm \pm 41.2 SE (range = 0.0 - 545.5 mm), and worm burden during the late collection period was 64.6mm \pm 11.5 SE (range = 19.0 - 196.5 mm). There was no significant change in worm burden recovered from stomach flushes across season (Kruskal-Wallis test: $H = 1.395$, $df = 2$, $P = 0.498$).

Nematodes from lizard cloacas

A total of 22 nematodes were collected from cloaca flushes and fecal pellets across the entire season, with 19 of 22 nematodes being recovered during the middle collection period. Only 3 nematodes were recovered during the late collection period. All nematodes were gravid females, with an average length of 17.14 mm \pm 0.70 SE (range 11.0 - 24.5 mm). This mean length was significantly greater than the mean length of gravid female nematodes from stomach flushes (Mann-Whitney U-test: $U = 2596$, $Z = 5.696$, $P < 0.0001$.) During the middle collection period, 8 out of 15 lizards (53.3% prevalence) harbored these 19 nematodes and during the late collection period, 3 out of 14 lizards (21.4% prevalence) harbored these 3 nematodes. A chi squared prevalence test revealed that there were significantly more lizards with nematodes in their cloacas during the middle collection period compared to the late collection period ($X^2 = 4.24$; $df = 1$, $P = 0.04$).

Prevalence of infection in *Pogonomyrmex* spp. ants

Approximately 6,000 *Pogonomyrmex* spp. ants were collected throughout the season (about 2,000 were dissected from each collection period, with 58,5 ants per pit-

trap cup), and only one ant was infected with one larval *S. phrynosoma*. This infected ant was collected during the early collection period.

DISCUSSION

The life cycle of *S. phrynosoma* is unique to other helminth parasites in that gravid females exit their lizard definitive host to die on the desert floor. Ant intermediate hosts are infected when this dead gravid female is foraged by adult harvester ants and fed to the brood of the colony. This study aimed to help describe this unique system by examining seasonal effects on *S. phrynosoma* in the lizard definitive host, *P. platyrhinos*, and in the ant intermediate host *Pogonomyrmex* spp. Parasite load variables were analyzed from lizard stomach flushes and cloacal sampling, both within collection period (early, middle and late) and across active season. *Pogonomyrmex* spp. ants were collected and dissected to determine the prevalence in this host across season. Many interesting patterns of infection dynamics in lizard hosts were observed, including the maintenance of a stable male population across season and the significant decrease in non-gravid female nematodes across season.

The number of male nematodes remained constant across season, yet the length of male nematodes showed a significant increase from early to middle to late collection period, indicating continual growth. As the number of male nematodes in lizard stomachs remained stable throughout the season, it seems that males stop accumulating in lizards after a set population has been reached. These males also may remain in lizards throughout the season, or for several years, as indicated by the increase in length of male nematodes throughout the season, and the absence of male nematodes in cloaca flushes

and with fecal pellets. These findings suggest a mechanism in *S. phrynosoma* that limits the number of male nematodes in a lizard's stomach. One hypothesis is that sex determination of these nematodes does not happen until larvae reach the lizard's stomach, and that a critical mass of male nematodes may set off cues that trigger incoming larval nematodes to become female. Environmental sex determination (ESD) is a mechanism used by some vertebrates such as fish and reptiles (Bull 1980, Conover and Kynard 1981) and invertebrates such as shrimp (Adams et al. 1987). More importantly, ESD is observed in invertebrate-parasitic nematodes (Christie 1929, Petersen 1972), but has never been described in vertebrate-parasitic nematodes, as these nematodes are known to exhibit chromosomal sex determination (Post 2005). The infection dynamics of female nematode populations in stomachs and cloacas of *P. platyrhinos* support this hypothesis.

During the early collection period, females, specifically non-gravid female nematodes, were more numerous in stomach flushes than any other sex category. These results were consistent with the findings of other authors who stated that there is generally a female bias in sex ratios of parasitic nematodes (Haukisalmi et al. 1996, Poulin 1997a, b, Stein et al. 2005), and May and Woolhouse (1993) suggested that female-biases are favored in polygamous parasite mating systems. But the number of nematodes, and more specifically, the number of non-gravid female and juvenile nematodes in lizard stomachs significantly decreased between early to late collection period. This decline in numbers of *S. phrynosoma* in *P. platyrhinos* may have been a result of fluctuating hormones or physiological stresses due to seasonal breeding but it seems, more importantly, that this seasonal change in parasite load was driven by the unique life cycle of *S. phrynosoma*. As indicated by the decrease in non-gravid female

nematodes throughout the season, these young females (possibly present immediately after host emergence from hibernation) mate with males early in the season and become gravid, jump-starting the parasite's seasonal life cycle. Although cloacal samples showed that gravid female nematodes were exiting lizard hosts during middle and late collection period, the concurrent conversion of non-gravid females to gravid kept the number of gravid females constant throughout the season. The absence of any increase in size of gravid females in stomach flushes across season suggests that these females remained in the lizard's stomach only long enough to become gravid, which Lee (1957) reported may be around 65 days, and immediately migrated toward the cloacas. The significantly greater length of cloacal nematodes compared to gravid females from stomach flushes indicates that a period of growth occurs as these gravid females move toward the cloaca before exiting with the host's feces.

The relative length of gravid females to other sex categories was consistent with the findings of Morand and Hugot (1998), who reported that females of oxyurid nematode species were consistently larger than males. Non-gravid females did not follow this pattern, most likely because as non-gravid females age, they mate and become gravid females. If the quota of male nematodes has been established in a lizard's stomach (see above) the sex category succession of incoming nematodes may be as follows: juvenile nematodes grow into non-gravid females, then, after mating, these non-gravid females become gravid females and after a period of growth, exit the lizard with fecal pellets.

Mean length of juvenile nematodes during the middle collection period was greater than that during the early collection period and the late collection period. The

increase in length of these juveniles from early to middle collection period may have been a result of juveniles growing in the lizards' stomachs, and the observed decrease in mean length of juveniles between middle and late collection period may be explained by acquisition of new infection (i.e., the newly-establishing juveniles are smaller, as they recently emerged from infected ants).

Although there was an apparent decrease in worm burden between the middle and late collection period, this decrease was not significant. Worm burden was defined as the sum of all of the nematodes' lengths in a lizard's stomach, which incorporates the variables of both nematode number and length. With a significant decrease in the number of female nematodes between the early and late collection periods, the absence in significant change in worm burden across season may be partially explained by the significant increase in length of male nematodes. It is possible that the exiting of female nematodes may have resulted in more space and resources available for use by male nematodes, which in turn could promote growth of these males. Even though there were fewer total nematodes in lizards' stomachs, male nematodes may have grown in the absence of the worm burden from other sex categories.

The progression of the *S. phrynosoma* life cycle after the exit of gravid females is unclear. Gravid female nematodes are available to foraging ants from middle to late collection period and, after being fed to and ingested by larval ants, the larval nematodes may overwinter within the broods of the ant colonies, becoming available again for infection of the definitive host after hibernation. With the extremely low prevalence of infection observed in the ant intermediate host, possibly due to inadequacies of sampling

method, it is still unclear when or how frequently lizards become infected with larval *S. phrynosoma*.

This study presents the first thorough investigation of host/parasite dynamics of *S. phrynosoma* in its hosts in the Alvord Basin. There were many interesting patterns of nematode sex distributions in lizards' stomachs across season, including the maintenance of a stable male nematode population as well as the decrease in non-gravid female nematodes across season. It seems that seasonal parasite load of *S. phrynosoma* in *P. platyrhinos* is dependent upon the timing of the life cycle of both hosts as well as of the nematode, and is especially driven by the movement of female *S. phrynosoma* through this system. Many questions have been raised as a result of this research, e.g., timing of infection in intermediate and definitive hosts, amount of time required for development in these hosts, and environmental cues affecting parasite life cycle. Multiple years of data would be helpful in addressing these questions, and would help to fully describe the dynamics of this interesting life cycle.

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Table 1. Mean number of *S. phrynosoma* per *P. platyrhinos* stomach flush collected during three collection periods of the 2008 active season.

Nematode sex category	Early (n = 13)		Middle (n = 15)		Late (n = 14)	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Male	5.2 \pm 1.07	0 - 12	5.4 \pm 1.59	0 - 20	4.36 \pm 0.97	0 - 15
Female	13.8 \pm 3.35	2 - 36	11.3 \pm 3.68	0 - 47	3.1 \pm 0.66	1 - 9
Non-gravid female	11.5 \pm 2.6	2 - 29	6.7 \pm 2.48	0 - 30	1.8 \pm 0.26	0 - 4
Gravid female	2.3 \pm 0.90	0 - 9	4.7 \pm 1.55	0 - 17	1.3 \pm 0.49	0 - 5
Juvenile	3.9 \pm 0.78	0 - 9	2.2 \pm 0.67	0 - 8	1.3 \pm 0.46	0 - 6
Total	23.0 \pm 4.84	7 - 54	18.9 \pm 5.52	0 - 66	8.7 \pm 1.60	2 - 26

Table 2. Mean *S. phrynosoma* length (mm) per collection period (irrespective of host) collected from *P. platyrhinos* stomach flushes during the active season of 2008 in the Alvord Basin of southeastern Oregon.

Nematode sex category	Early		Middle		Late	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Male	6.0 \pm 0.21 (n = 68)	3.5 - 11.5	6.5 \pm 0.13 (n = 81)	4 - 10	8.2 \pm 0.26 (n = 61)	4.5 - 13.5
Non-gravid female	6.2 \pm 0.21 (n = 150)	2.5 - 16	6.2 \pm 0.29 (n = 100)	2.5 - 19	6.1 \pm 0.50 (n = 24)	4 - 15
Gravid female	11.5 \pm 0.62 (n = 30)	7 - 23	12.3 \pm 0.40 (n = 70)	8 - 22	11.3 \pm 0.23 (n = 18)	10 - 13
Juvenile	2.9 \pm 0.14 (n = 42)	2 - 5.5	3.8 \pm 0.24 (n = 33)	2 - 8	2.9 \pm 0.14 (n = 18)	2 - 4

Table 3. Mean worm burden ($\sum L$) (mm) of *S. phrynosoma* per *P. platyrhinos* stomach flush collected during three collection periods of the active season of 2008.

Nematode sex category	Early (n = 13)		Middle (n = 15)		Late (n = 14)	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Total	139.0 \pm 32.74	32 - 348	142.2 \pm 42.60	0 - 545.5	64.6 \pm 11.93	19 - 196.5

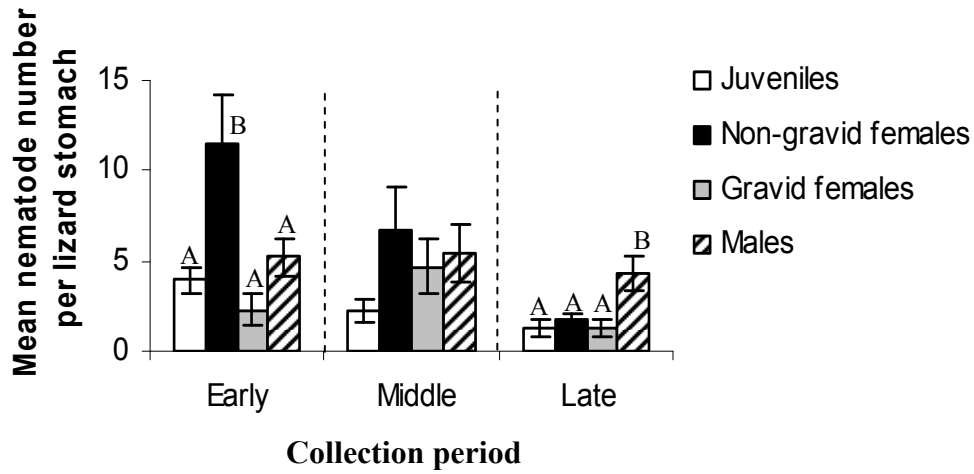


Figure 1. Distribution of *S. phrynosoma* in *P. platyrhinos* stomach flushes within collection period. Mean nematode number per lizard stomach is shown for the three collection periods (early, n = 13; middle, n = 15; late, n = 14). Letters above bars represent significant differences within each collection period. During the early collection period there were significantly more non-gravid female nematodes than juveniles, gravid females or males. During the late collection period there were significantly more male nematodes than juveniles, non-gravid females and gravid females. (See text for statistical analyses.) Numbers within bars represent sample size of lizard hosts. Error bars are standard error.

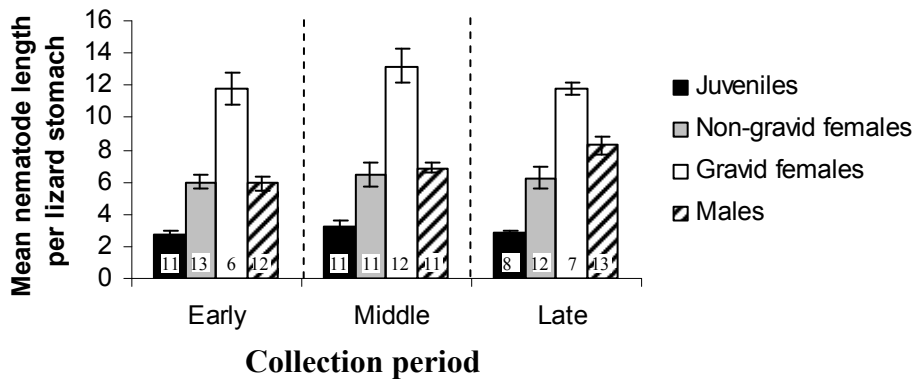


Figure 2. Distribution of *S. phrynosoma* length (mm) in *P. platyrhinos* stomach flushes within collection period. Bars represent the average mean length of nematodes per stomach flush. (See text for statistical analyses.) Numbers within bars represent sample size of lizards containing particular nematode sex category. Error bars are standard error.

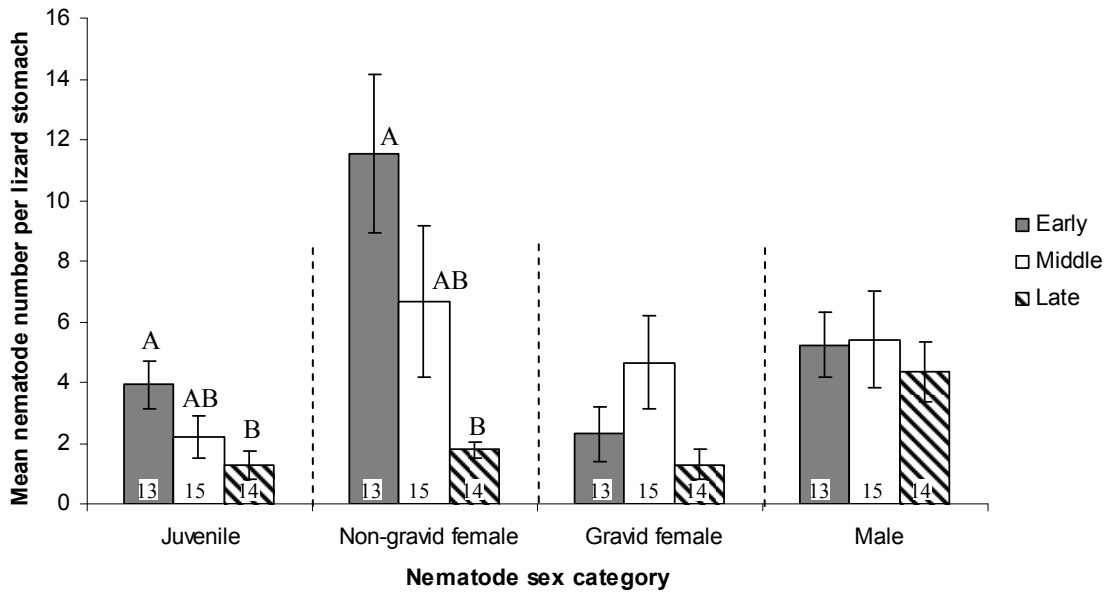


Figure 3. *Skrjabinoptera phrynosoma* population structure in *P. platyrhinos* stomach flushes across season. Mean number of nematodes for each sex category is shown across three collection periods. Letters above bars represent significant differences within sex category. There were significantly more juvenile nematodes in lizard stomachs during the early collection period as compared to the late collection period and there were significantly more non-gravid female nematodes in lizard stomachs during the early collection period as compared to the late collection period (See text for statistical analyses). Numbers within bars represent sample size of lizard hosts. Error bars are standard error.

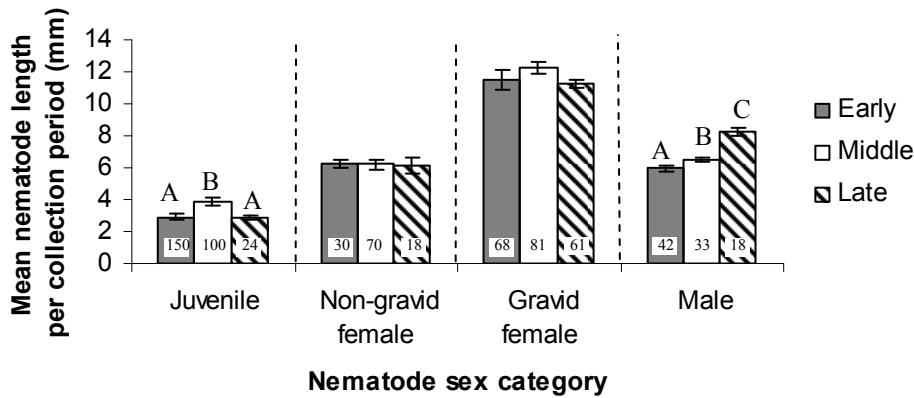


Figure 4. Mean lengths (mm) of *S. phrynosoma* across season, irrespective of host. The mean length of nematodes for each sex category is shown across three collection periods (early, middle and late). Letters above bars represent significant differences within sex category. The length of juvenile nematodes during the middle collection period was significantly greater than that the early collection period and the length of juveniles in the late collection period was significantly less than that in the middle collection period. Length of male nematodes in middle collection period was significantly greater that that in early collection period, and length of males in late collection period was significantly greater than that in middle collection period. (See text for statistical analyses.) Numbers in bars represent sample size of nematodes in each category. Error bars are standard error.

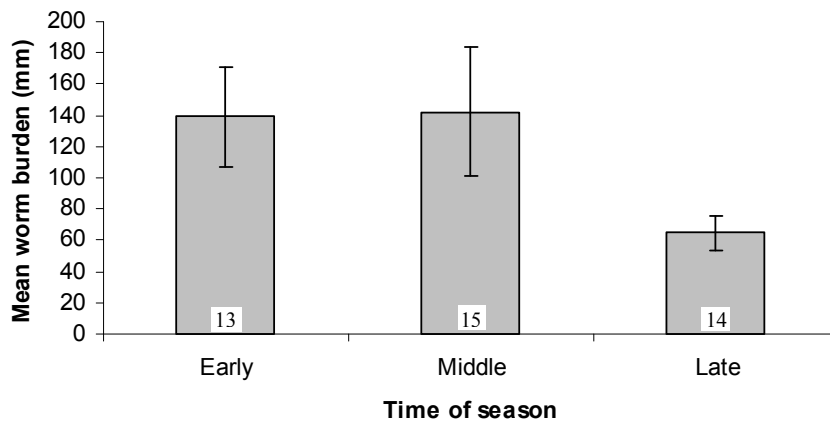


Figure 5. Mean worm burden (ΣL) (mm) of *S. phrynosoma* in *P. platyrhinos* stomach-flushes during three collection periods (early, middle and late). There was no significant change in worm burden across season. (See text for statistical analyses). Numbers in bars represent sample size of lizard hosts. Error bars are standard error.

CHAPTER 2

HOST EFFECTS ON THE INFECTION DYNAMICS OF *SKRJABINOPTERA*
PHRYNOSOMA (ORTLEPP) SCHULZ, 1927, A PARASITIC NEMATODE OF
HORNED LIZARDS

ABSTRACT

The parasitic nematode *Skrjabinoptera phrynosoma* (Ortlepp) Schulz, 1927 was collected from the desert horned lizard *Phrynosoma platyrhinos* Gerard, 1852 to describe the effect of host sex and size (SVL) on parasite load. Nematodes were collected from stomach flushes, cloaca flushes and fecal pellets during three collection periods throughout the active season of 2008. Parasite load variables (which included number of nematodes per lizard stomach, length of those nematodes, and total worm burden in stomachs) were compared in male versus female lizards and also correlated with lizard SVL. Parasite load variables of different nematode sex categories were analyzed within each collection period. Host sex did not affect parasite load, but there were correlations between host SVL to nematode length and number. During the early collection period, host SVL was positively correlated with length of non-gravid female nematodes and juvenile nematodes. During the late collection period, there was a negative relationship between lizard SVL and number of gravid female nematodes. Cloacal sampling yielded only large gravid female nematodes. These effects of lizard SVL on parasite load are most likely driven by the succession of development of these nematodes within the lizards' stomachs. It is proposed that newly-establishing nematodes (juveniles) can develop into females,

mate, and exit larger lizards faster because of more space and resources available in the larger stomachs.

INTRODUCTION

Parasite load is often a function of host characteristics such as sex and size. Greater parasite load in one host sex over another, or 'sex-biased parasitism', has been most commonly observed in male hosts (Poulin 1996, Schalk and Forbes 1997), but is seen in females as well (McCurdy et al. 1998). Several authors (Zuk and McKean 1996, McCurdy et al. 1998, Moller et al. 1998) outline possible influences of sex biases on parasitism, including hormonal immunosuppression, stresses of mating systems, and behavior. Testosterone is believed to suppress the immune system of animals (Schalk and Forbes 1997, Casto et al. 2001), and several authors have demonstrated an increased parasite load in male hosts due to elevated testosterone (Kiyota et al. 1984, Nakanishi et al. 1989, Tiuria et al. 1994). Physical stresses caused by various mating systems, such as defense of larger territories or courtship by males during breeding season, may also compromise the immune system in a potential host (Zuk and McKean 1996). Finally, as a result of behavioral differences between potential host sexes, exposure to parasites or vectors may vary between host sexes (McCurdy et al. 1998). These influences do not act independently, and sex-biased parasitism probably results from a combination of these factors.

Parasite load also may be influenced by host size, which in turn may be related to age of host. Sorci (1996) demonstrated that haemogregarine load (percent parasitized blood cells) in the lizard host showed a bell curve, with mid-sized lizards having the

highest load. Larger hosts had experienced changes in their feeding behavior or physiology or had achieved some immune defense to parasites. As modeled by Anderson and Gordon (1982), highly pathogenic parasites may kill off young hosts, leaving older/larger hosts with a low parasite load. Less pathogenic parasites slowly accumulate in hosts, causing older/larger hosts to have a higher parasite load than younger hosts. On the other hand, low parasite-induced host mortality can result in a high parasite aggregation within hosts (Anderson and Gordon 1982, Sorci 1996, Duerr et al. 2002). As suggested by McCurdy et al. (1998), host size may also affect parasite load simply because larger hosts provide more surface area and niches for parasites.

The present study addresses parasite load of *Skrjabinoptera phrynosoma* (Spirurida: Physalopterinae) (Ortlepp) Schulz, 1927 in the desert horned lizard *Phrynosoma platyrhinos* Gerard, 1852, and the effect of host sex and size on this parasite load. The life cycle of the parasitic nematode *S. phrynosoma* is unique in that instead of passing eggs in the feces of the definitive host, as most physalopterines do, whole gravid females of *S. phrynosoma* exit with feces of the lizard definitive host. The larval ant intermediate host is infected after the dead gravid female nematode is foraged by adult harvester ants and fed to the brood of the colony. The larval *S. phrynosoma* molt within the developing ant and reach the infective third larval stage when the ant reaches adulthood. The life cycle is completed when the foraging adult ant is eaten by the *Phrynosoma* spp. lizard and the nematode molts into an adult in the lizard's stomach. Like other members of the subfamily Physalopterinae, *S. phrynosoma* attach to the stomach mucosa of the definitive host when not feeding, and detach to feed on host stomach contents (Anderson 1992). Although *S. phrynosoma* is commonly recovered in

P. platyrhinos (Babero 1967; Grundmann 1959; Waitz 1961), this particular host/parasite system has not been studied, and little is known of the infection dynamics of *S. phrynosoma* in any host.

The purpose of this study was to determine the possible effects of host characteristics, specifically host sex and size, on the parasite load of *S. phrynosoma* in *P. platyrhinos*, a sexually dimorphic horned lizard found in the northern arid regions of North America (Pianka and Parker 1975). To address effects of host size and sex on parasite load in *P. platyrhinos*, 1.) Parasite load (nematode number, nematode length, and total worm burden) was compared in male and female lizards and 2.) the relationship between size of lizard host (SVL) and parasite load was analyzed.

METHODS

Lizard Measurements

Data were collected three times throughout the active season of 2008 on a 400 × 400 meter plot in the Alvord Basin in southeastern Oregon: early (soon after emergence from hibernation and during breeding; May 16 - June 2; n = 13), middle (during egg laying; June 25 – July 15; n = 15), and late collection period (preparation for hibernation; August 1 – 15; n = 14). Lizards were sexed, weighed, measured (snout-vent length (SVL)), and marked for identification to avoid resampling during subsequent collection periods.

Nematode collection

To retrieve *S. phrynosoma* from the digestive tract of *P. platyrhinos*, lizards were stomach-flushed and cloaca-flushed within 1-5 days of capture. Lizards were stomach

flushed with ambient temperature standard Ringer's solution (NaCl, 0.66%; KCl, 0.015%; CaCl₂, 0.015%; NaHCO₃, 0.02%) to retrieve stomach parasites, and stomach contents were preserved in 75% glycerin alcohol in 20 mL vials. Cloaca flushing also was used to assess parasite infection in the colon and cloaca, following the methods of Cannon (2003) and Mader (1996). Ringer's solution was flushed into cloaca and aspirated to retrieve nematodes and the aspirate was preserved with glycerin alcohol in a 20 mL vial. Fecal pellets also were collected, and nematodes expelled along with fecal pellets were preserved in glycerin alcohol.

Nematode analysis

Nematodes from stomach and cloaca flushes and fecal pellets were counted, measured (mm) and sexed. Sex of nematodes was determined by presence of wing-like caudal alae in males and a curling of the caudal end in females (Babero and Kay 1967). Females were also determined to be gravid or non-gravid by the presence of visible egg packets in gravid females (Lee 1957). Nematodes that did not have apparent male or female characteristics were classified as juvenile nematodes. Nematode worm burden, or total length of all nematodes in a stomach flush ($\sum L$), was calculated.

Statistical Analysis

To determine possible effect of lizard sex on nematode number, average nematode length, and worm burden, these variables were compared in male versus female lizards. Nematode data were log transformed where necessary, and t-tests were performed on parasite load variable in male vs. female lizards. Non-parametric Mann-Whitney U-tests were run on data that could not be normalized.

To determine the relationship between lizard SVL and stomach nematode load, SVL was correlated with nematode number, average nematode length, and worm burden from stomach flushes. Data were log transformed where necessary, to use Pearson's product moment on parametric data, and Spearman's rank correlation was used to analyze non-parametric data. Data for total number of nematodes was analyzed, and nematode data was also subcategorized by sex. Since there was a change in nematode number and length across season (see Chapter 1), these analyses were performed on data for each individual collection period.

There was a small sample size of lizards with cloacal nematodes (see Chapter 1), and a t-test was performed to compare SVL of lizards with cloacal nematodes versus lizards without cloacal nematodes. Additionally, lizard SVL and cloacal nematode length was correlated. Sample size was too small to compare cloacal nematode data in male and female lizards.

All analyses were performed using the statistical program JMP (SAS Institute Inc. 2001), and graphs were produced using Microsoft Excel (Microsoft Corporation 2003).

RESULTS

When measures of parasite load (nematode number, length, and worm burden) were compared between male and female lizards, there was no effect of lizard sex during any part of the season ($P > 0.12$ for all analyses), therefore male and female lizards were grouped together for the remaining analyses.

There were no significant relationships between lizard SVL and nematode number in stomach flushes during the early and middle collection period, but during the late

collection period, there was a negative relationship between lizard SVL and gravid female number (Spearman's Rho = -0.5841, $n = 14$; $P = 0.0283$, Fig. 1). This was driven by an absence of any gravid females in the five largest lizards. During the early collection period, there was a positive relationship between lizard SVL and average length of non-gravid female ($r = 0.7049$, $n = 13$, $P = 0.0071$) and juvenile nematodes ($r = 0.6994$, $n = 11$, $P = 0.0166$, Fig. 2, 3). There were no relationships between lizard SVL and worm burden ($P > 0.15$: all analyses for all collection periods).

A total of 22 nematodes were collected from cloaca flushes and fecal pellets across the entire season (see Chapter 1). All nematodes were gravid females with an average length of $17.14 \text{ mm} \pm 0.70 \text{ SE}$ (range 11.0 - 24.5 mm). A t-test of SVL of lizards with cloacal nematodes versus lizards without cloacal nematodes showed that there was no significant difference during the SVL of these two groups ($t = -1.245$, $df = 27$, $P = 0.2237$). There was also no significant relationship between lizard SVL and length of cloacal nematodes ($r = -0.1918$, $n = 11$, $P = 0.5722$).

DISCUSSION

Little is known about the host/parasite dynamics of *Skrjabinoptera phrynosoma* and *Phrynosoma platyrhinos*. This study examined the relationship between host characteristics (sex, SVL) and parasite load (number, length and worm burden). Although there are several sex-specific differences in *Phrynosoma* lizards (Pianka and Parker 1975, Wone and Beauchamp 2003, Moeller et al. 2005, Wack et al. 2007), there was no sex-biased parasitism observed with number of nematodes, average length of nematodes, or worm burden in lizards.

There were few significant relationships between lizard SVL and parasite load variables. When lizard SVL was correlated with average lengths of nematodes in different sex categories, the only significant relationships occurred during the early collection period. Lizards with greater SVL had both longer non-gravid female and juvenile nematodes. Furthermore, during the late collection period, lizards with a greater SVL had fewer gravid female nematodes. In fact, the five largest lizards did not have any gravid female nematodes (Fig. 1).

These results imply that in larger lizards growing nematodes may be able to develop and mature at a faster rate, resulting in the hastened departure of the gravid female nematodes with the lizards' feces. This accelerated growth rate may be due to a greater amount of space within larger lizards, which provides more room for attachment, or more niches for parasites, as suggested by McCurdy et al. (1998). Further, since these nematodes feed on host stomach contents instead of host tissue (Anderson 1992), this accelerated growth may be due to an increased dietary intake by larger lizards, providing more nutrients not only for the host, but also for the parasites. Increased dietary intake in the stickleback fish *Gasterosteus aculeatus*, was associated with increased worm burden of the cestode *Schistocephalus solidus* (Barber 2005). An increase in host food intake was responsible for the significant positive relationship between host growth and parasite burden. When *P. platyrhinos* fecal pellet data from 2006 were analyzed, it was determined that *P. platyrhinos* with a greater SVL consumed marginally more ants than lizards with a smaller SVL ($r = 0.3288$, $n = 35$, $P = 0.0538$) (Anderson, unpublished).

The lack of relationship between lizard SVL and length of cloacal nematodes implies that, irrespective of host size, gravid females remain in hosts until an optimal

length has been attained, before exiting the host. This is supported by the greater number of gravid female nematodes in smaller lizards later in the season (Fig. 1).

This research presents the first thorough investigation of host characteristics and parasite load dynamics in the life cycle of *S. phrynosoma*, and interesting relationships between lizard SVL and parasite load variables have been described. Although these relationships suggest varying growth rates of nematodes in lizards of different sizes, further investigation is needed to fully describe these relationships and to determine the causes of these relationships. There are many unknown variables in the parasite/host dynamics of *S. phrynosoma* in the Alvord Basin, but this research serves as a preliminary analysis to help describe this unique life cycle.

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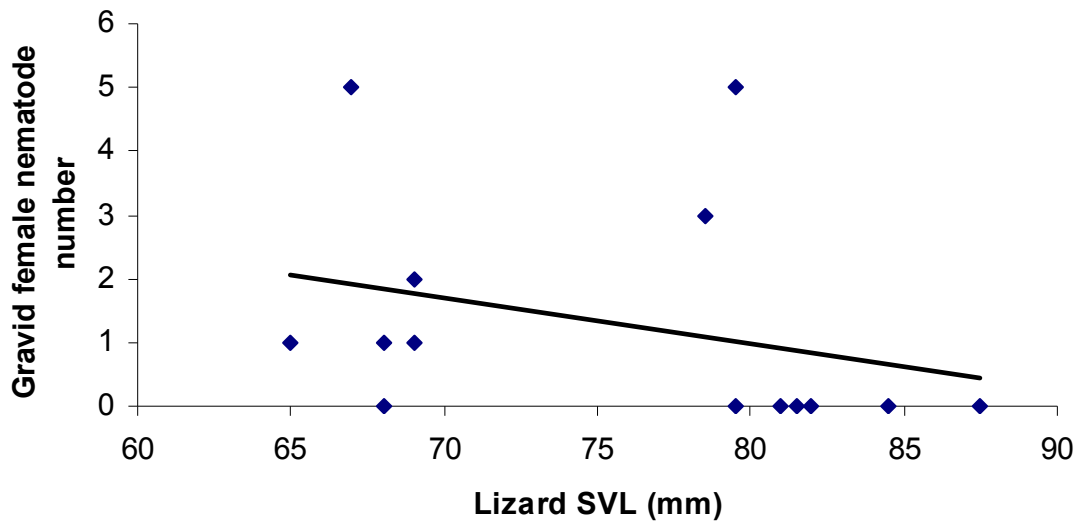


Figure 1. Correlation between *P. platyrhinos* SVL (mm) and number of gravid female *S. phrynosoma* in lizard stomachs during the late collection period. There was a negative relationship between lizard SVL and number of gravid female nematodes (see text for statistical analyses). Line shown is a trendline.

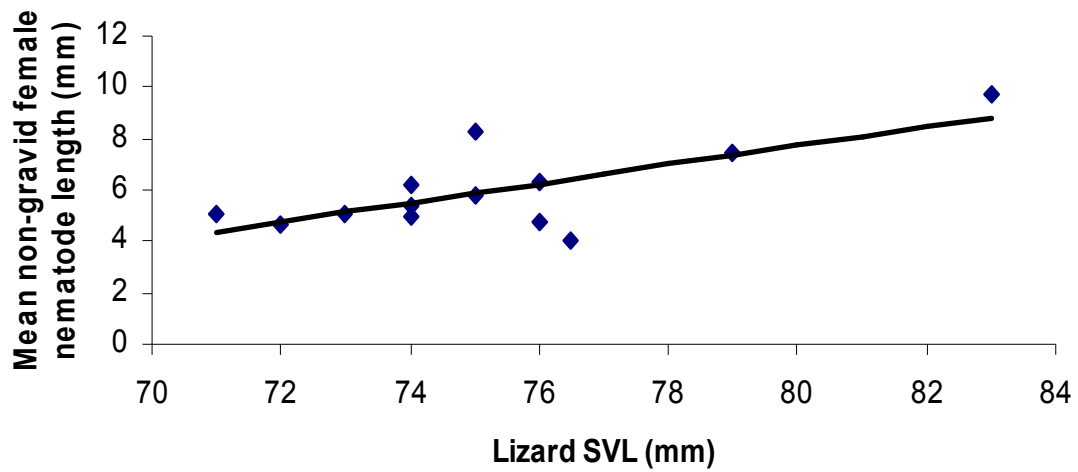


Figure 2. Correlation between *P. platyrhinos* SVL (mm) and mean non-gravid female *S. phrynosoma* length (mm) in lizard stomachs during the early collection period. There was a positive relationship between lizard SVL and mean length of non-gravid female nematodes (see text for statistical analyses). Line shown is a trendline.

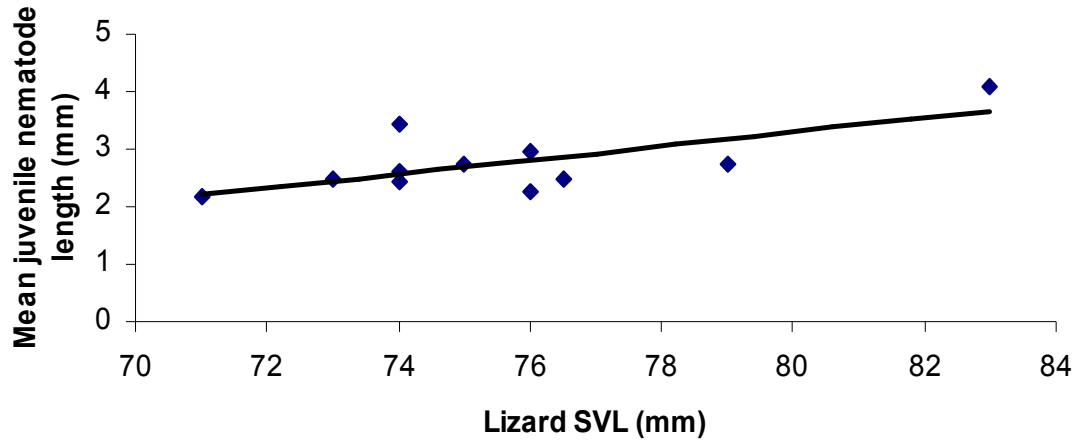


Figure 3. Correlation between *P. platyrhinos* SVL and mean juvenile *S. phrynosoma* length (mm) in lizard stomachs during the early collection period. There was a positive relationship between lizard SVL and mean length of juvenile nematodes (see text for statistical analyses.) Line shown is a trendline.

APPENDIX

Appendix Table 1. Comparisons of total *S. phrynosoma* number in male vs. female *P. platyrhinos* during three collection periods, and comparisons of total worm burden (ΣL) (mm) in male vs. female lizards during three collection periods

Collection period	Total nematode number per lizard		
	<i>P</i> -value	<i>t</i> -value	df
Early	0.2911	0.416	11
Middle	0.4561	-0.768	13
Late	0.6972	-0.399	12

Collection period	Nematode Worm Burden (ΣL)		
	<i>P</i> -value	<i>t</i> -value	df
Early	0.1842	1.417	11
Middle	0.1756	-1.432	13
Late	0.5948	-0.546	12

Appendix Table 2. Comparisons of mean *S. phrynosoma* length (mm) per *P. platyrhinos* in male vs. female lizards (all collection periods combined)

Nematode sex category	Mean nematode length per lizard		
Male	<i>P</i> = 0.1214	<i>t</i> = 1.588	df = 34
Non-gravid female	<i>P</i> = 0.8221	<i>Z</i> = 0.225	Female liz. N = 15 Male liz. N = 21
Gravid female	<i>P</i> = 0.6364	<i>Z</i> = -0.473	Female liz. N = 10 Male liz. N = 15
Juvenile	<i>P</i> = 0.1412	<i>Z</i> = -1.471	Female liz. N = 11 Male liz. N = 19

Appendix Table 3. Correlations of *P. platyrhinos* host SVL (mm) to mean *S. phrynosoma* number in stomach flushes during three collection periods. Asterisk shows significant *P*-value.

Nematode sex category	Early (n = 13)		Middle (n = 15)		Late (n = 14)	
	<i>P</i> -value	Test statistic	<i>P</i> -value	Test statistic	<i>P</i> -value	Test statistic
Male	0.6880	Spearman's Rho: 0.1234	0.3608	Spearman's Rho: 0.254	0.3224	Correlation: 0.2855
Female	0.9461	Spearman's Rho: -0.0208	0.6916	Spearman's Rho: 0.1118	0.7319	Spearman's Rho: -0.1007
Non-gravid female	0.7482	Correlation: 0.0988	0.3888	Spearman's Rho: 0.2400	0.4511	Spearman's Rho: 0.2194
Gravid female	0.2464	Spearman's Rho: 0.3463	0.6810	Spearman's Rho: -0.1158	0.0283*	Spearman's Rho: -0.5841
Juvenile	0.8711	Correlation: -0.0500	0.9111	Spearman's Rho: -0.0329	0.6285	Spearman's Rho: -0.1419
Total	0.9856	Spearman's Rho: -0.0056	0.3552	Correlation: 0.2270	0.5100	Correlation: 0.1924

Appendix Table 4. Correlations between *P. platyrhinos* SVL (mm) and mean *S. phrynosoma* length (mm) per stomach flush during three collection periods. Asterisks show significant *P*-values.

Nematode sex category	Early			Middle			Late		
	<i>P</i> -value	correlation	n	<i>P</i> -value	correlation	n	<i>P</i> -value	correlation	n
Male	0.2183	0.3836	12	0.1483	-0.4662	11	0.8825	0.0456	13
Non-gravid female	0.0071*	0.7049	13	0.1201	-0.4967	11	0.2241	-0.5271	7
Gravid female	0.8464	-0.1028	6	0.7425	-0.1062	11	0.2817	-0.3386	12
Juvenile	0.0166*	0.6994	11	0.4845	-0.2362	12	-	-	-

Appendix Table 5. Change in *S. phrynosoma* number per *P. platyrhinos* stomach flush across season and between collection periods. Asterisks denote significant *P*-values.

Nematode sex category	Across season			Early vs. Mid			Mid vs. Late			Early vs. Late		
	<i>P</i> -value	Test statistic	df	<i>P</i> -value	Test statistic	n	<i>P</i> -value	Test statistic	n	<i>P</i> -value	Test statistic	n
Male	0.7800	F ratio = 0.250	2, 39	-	-	-	-	-	-	-	-	-
Female	0.0149*	H = 8.407	2	0.3094	Z = 1.017	Early n = 13 Mid n = 15	0.1599	Z = -1.405	Mid n = 15 Late n = 14	0.0018*	Z = 3.130	Early n = 13 Late n = 14
Non-gravid female	0.0018*	H = 12.636	2	0.0548	Z = 1.920	Early n = 13 Mid n = 15	0.3741	Z = -0.889	Mid n = 15 Late n = 14	< 0.0001*	Z = 3.891	Early n = 13 Late n = 14
Gravid female	0.1378	H = 3.963	2	-	-	-	-	-	-	-	-	-
Juvenile	0.0167*	H = 8.190	2	0.0527	Z = 1.937	Early n = 13 Mid n = 15	0.33302	Z = -0.974	Mid n = 15 Late n = 14	0.0066*	Z = 2.175	Early n = 13 Late n = 14
Total	0.0797	H = 5.059	2	0.3207	Z = 0.993	Early n = 13 Mid n = 15	0.4569	Z = -0.744	Mid n = 15 Late n = 14	0.0130*	Z = 2.485	Early n = 13 Late n = 14

Appendix Table 6. Change in mean *S. phrynosoma* length (mm) (irrespective of host) across season and between collection periods. Asterisks denote significant *P*-values.

Nematode sex category	Across season			Early vs. Mid			Mid vs. Late			Early vs. Late		
	<i>P</i> -value	Test statistic	df	<i>P</i> -value	Test statistic	n	<i>P</i> -value	Test statistic	n	<i>P</i> -value	Test statistic	n
Male	0.0001*	H = 47.980	2	0.0017*	Z = -3.130	Early n = 68 Mid n = 81	0.0001*	Z = 5.243	Mid n = 61 Late n = 81	0.0000*	Z = 6.123	Early n = 68 Late n = 61
Non-gravid female	0.9838	H = 0.0327	2	-	-	-	-	-	-	-	-	-
Gravid female	0.5462	H = 1.210	2	-	-	-	-	-	-	-	-	-
Juvenile	0.0003*	H = 16.019	2	0.0002*	Z = 3.745	Early n = 42 Mid n = 33	0.0051*	Z = -2.801	Mid n = 18 Late n = 33	0.5410	Z = 0.611	Early n = 42 Late n = 18