PARASITE COMMUNITY STRUCTURE AND REPRODUCTIVE STRATEGIES OF *GYRINICOLA BATRACHIENSIS* IN TADPOLE STAGES OF ANURANS FROM NORTH CENTRAL OKLAHOMA

By

CHELCIE CIERRA PIERCE

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PARASITE COMMUNITY STRUCTURE AND REPRODUCTIVE STRATEGIES OF *GYRINICOLA BATRACHIENSIS* IN TADPOLE STAGES OF ANURANS FROM NORTH CENTRAL OKLAHOMA

Thesis Approved:

Dr. Matthew Bolek

Thesis Adviser

Dr. Monica Papeş

Dr. Andrew Dzialowski

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Abstract: Amphibians are commonly infected with parasites. As a result, they have been used as model systems to investigate parasite community structure in vertebrate hosts. Multiple factors have been identified to influence amphibian parasite community structure, including host size, habitat, and diet, as well as parasite transmission strategies. However, currently little information is available on how parasite communities are structured in tadpole stages of amphibians. The objectives of this thesis were to examine the parasite community structure of tadpoles of sympatric and syntopic anuran species from north central Oklahoma. Specifically, I investigated whether species-specific differences in tadpole size, feeding strategies, and habitat-use would affect parasite community structure. Additionally, I investigated the distribution and reproductive strategies of the tadpole pinworm, *Gyrinicola batrachiensis*, in tadpoles of five species of co-occurring anurans which differed in their time to metamorphosis. My data indicate that tadpole size is a primary factor in determining parasite abundances and intensities. However, after controlling for host size, parasite life history and host species were major factors in influencing parasite community structure within tadpole stages of anurans. Additionally, my study suggests that host species-specific differences, but not necessarily time to metamorphosis, play a major role on the growth, development, and reproductive strategies exhibited by *G. batrachiensis* in different amphibian species. This work provides evidence that parasite communities of larval anurans differ in their structure from adult anurans. However, factors such as host size, diet, and habitat, as well as parasite transmission strategies, appear to play an important role in structuring parasite communities within anuran tadpoles.

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CHAPTER I

OVERVIEW

Parasitism is one of the most common ecological relationships in the biological world (Bush et al., 2001). It has been estimated that roughly 50% of all plant and animal species are parasitic at some point in their life cycle (Price, 1980) and almost all plant and animal species serve as hosts to parasites (Bush et al., 2001). However, most ecological studies in the past have focused on free-living organisms, with little attention placed on organisms such as parasites. More recently, amphibian parasites have received considerable attention in the ecological literature. Recent amphibian declines and extinctions have been documented around the world and helminth parasites (worms), particularly trematodes, have been linked to some of these declines (Johnson et al., 2002). Many recent comparative studies on amphibian helminth parasites have provided baseline data for the distribution, demography, host specificity, and life history of amphibian helminth parasites (Goater et al., 1987; Goldberg et al., 1996; Barton, 1999; McAlpine, 1997; Bolek and Coggins, 2003; Yoder and Coggins, 2007). However, all of these studies have focused on adult amphibian parasites and their community structure, and little information is available on parasites of larval stages, specifically of larval anurans (Rhoden and Bolek, 2011, 2012, 2015).

To the parasite, the amphibian host represents a resource and habitat where the parasite can grow and reproduce. Once produced, reproductive stages are released into the environment, where they undergo development, and then must find their way into another host. Therefore, unlike most free-living organisms, a major problem for parasites is to find the correct host to propagate the next generation and complete the life cycle. Parasite life cycles and their transmission strategies can be classified into two categories: heteroxenous and monoxenous (Esch et al., 1990). Monoxenous parasites are described as having simple life cycles, with only one host, the definitive host, where the parasite reaches sexual maturity and reproduces (e.g., monogeneans and some nematodes). Heteroxenous parasites, on the other hand, are described as having complex life cycles, utilizing at least one other host than the definitive host (e.g., trematodes and most cestodes). Populations and communities of helminth parasites can then be studied at multiple levels of hierarchical organization. Populations have been defined to consist of one species of parasite, while communities are composed of all or multiple species of parasites. Hierarchical organization is then defined as 1) infrapopulation and infracommunity- a population or community of parasites in/on an individual host; 2) component population and component community- all the infrapopulations and infracommunities of parasites in a population of hosts in a given habitat; and 3) suprapopulation and compound community- all the parasites of a given species (population) and all the parasites species (community), in all stages of development including free-living stages, and all stages within all hosts in an ecosystem (Bush et al., 2001).

Helminth community structure within a host can then vary from isolationist to interactive and anywhere in between (Esch et al., 1990; Bush et al., 2001). Isolationist communities are defined as depauperate, lacking in number and variety of species, and structured by random events. In contrast, interactive communities are species rich and interactions and competition among different parasite species is assumed to play an important role in the structure of these communities (Esch et al., 1990).

Interspecific variation in host diet, life history, and body size among sympatric species of amphibians offer an excellent comparative approach for studies on helminth community structure (Goater et al., 1987; Goldberg et al., 1996; Barton, 1999; McAlpine, 1997; Bolek and Coggins, 2003; Yoder and Coggins, 2007). Recent comparative studies on amphibian parasite life cycles, recruitment, and community structure in anuran hosts by Bolek and Coggins (1998, 2000, 2001, 2003), Hardin and Janovy (1988), Muzzall and coworkers (Muzzall, 1991; Muzzall and Peebles, 1991; Gilliland and Muzzall, 1999; Muzzall et al., 2001), Snyder and Janovy (1994, 1996), and McAlpine (1997), Bolek and Janovy (2007a, b, 2008), Bolek et al., (2009, 2010), Vhora and Bolek (2013, 2015) and Stigge and Bolek (2015) have provided baseline data on the distribution, demography, field host specificity, and life history of amphibian parasites. These studies indicate that host size, diet, and habitat all play important roles in structuring anuran helminth communities. For example, parasite communities of aquatic frogs are dominated by digenetic trematodes with complex life cycles and acquired by ingesting aquatic or semi-aquatic intermediate hosts; whereas the parasite communities of terrestrial anurans are dominated by nematodes with simple life cycles which are acquired directly from the soil. In contrast, arboreal and fossorial anurans are usually less predictable in the

composition of nematodes and digenetic trematodes in their helminth communities and their community composition is determined by the amount of time each species spends in an aquatic or terrestrial environment (Bolek and Coggins, 1998, 2000, 2001, 2003; Vhora and Bolek, 2013).

These studies also indicate that within individual anuran species, newly metamorphosed and juvenile anurans are less commonly infected with parasites than are larger adult frogs, indicating that size also plays a role in structuring anuran helminth communities. Numerous hypotheses have been advanced for the lack of parasites in small anurans and include such causal factors as lack of time for exposure to parasites, a small body size, which affects the surface area available for skin-penetrating parasites, and small gape size, which affects the size of potential intermediate hosts that can be ingested by these frogs.

As with anuran size, diet has also been implicated in playing a role in structuring parasite communities in amphibian hosts. For example, toads are active foragers, specializing on ants and beetles, while true frogs are sit-and-wait gape-limited predators feeding on a variety of invertebrate and vertebrate prey that can fit into their gape size (Bolek and Coggins, 2000, 2001, 2003; Vhora and Bolek, 2015). Thus, true frogs have a broader diet and tend to be infected with more helminth species which use intermediate hosts, while toads are dominated by direct skin-penetrating helminths with direct life cycles and their diet of ants and beetles contributes little to their helminth recruitment. Ultimately, adult amphibian helminth communities have been shown to be highly variable, depauperate, and noninteractive (Aho, 1990). However, the aforementioned studies only considered metamorphosed and/or adult amphibians, and as a result we

know almost nothing about how helminth communities are structured in tadpole stages of anurans (Rhoden and Bolek, 2012, 2015).

Tadpoles, which are the ephemeral, feeding, non-reproductive larvae of anurans, differ significantly from metamorphosed anurans in their biology (McDiarmid and Altig, 1999). Temperate-zone anuran tadpoles are found in aquatic habitats, where they feed on suspended and/or epibenthic algae and incidentally on zooplankton (Seale, 1980). In the United States and Canada, frogs with free-living, feeding tadpoles include 103 species in 20 genera and 10 families (Altig and McDiarmid, 2015). Tadpole stages of most of these anuran species live in non-flowing water, such as ponds and lakes, although a few species occur in slow flowing streams and rivers. Tadpoles of most North American anuran species are aquatic; however, tadpole stages of different species of frogs and toads occupy different habitats and depths within ponds and lakes suggesting that they partition their environment as do adult anuran species. For example, Heyer (1976) examined habitat partitioning among tadpoles of different anuran species from a pond in Virginia. His study showed that tadpoles of American toads, *Bufo americanus*, and spring peepers, *Pseudacris crucifer*, were distributed at the surface of the water column; tadpoles of Cope's gray treefrogs, *Hyla chrysoscelis*, and wood frogs, *Rana sylvatica*, were distributed in mid-water; whereas tadpoles of green frogs, *Rana clamitans*, occupied the bottom of the water column. These observations suggest that habitat may also play a major role in structuring parasite communities in larval stages of anurans.

Among anuran species, tadpole stages also vary greatly in their body size and time to metamorphosis. For example, tadpoles of American bullfrogs, *Rana catesbeiana*, can take up to three years before metamorphosing and these tadpoles can reach a

maximum length of 190 mm before metamorphosis (Bolek and Janovy, 2004). On the other hand, tadpoles of Couch's spadefoot toads, *Scaphiopus couchii* can metamorphose in as little as 7-16 days, and Oak toads, *Bufo quercicus*, only grow to 10-19 mm in total length before metamorphosis (Altig and McDiarmid, 2015). Additionally, tadpoles are completely aquatic and predominantly feed on algae, leading to a dramatically different body morphology and digestive system compared to those found in predatory metamorphosed and adult anurans. As a result, tadpoles have been considered vertebrate analogs to larvae of holometabolous insects (McDiarmid and Altig, 1999).

Despite numerous studies on ecological differences among tadpole stages of sympatrically distributed anuran species (Wilbur, 1972, 1976, 1980; Wilbur and Collins, 1973; Heyer, 1976; McDiarmid and Altig, 1999; Seale and Beckvar, 1980), few investigators have examined larval anuran stages for parasites (Adamson, 1981a, b, c; Hamann and Gonzalez, 2009; Rhoden and Bolek, 2011). More importantly, amphibian declines and extinctions have been documented around the world and some species of parasites infecting tadpoles have been linked to some of these declines (Holland, 2009, 2010; Johnson and McKenzie, 2009; Schotthoefer et al., 2003). However, we know almost nothing about how parasite communities are structured in this life stage of amphibians. In fact, only three studies on helminth community structure exist in tadpoles (Kehr and Hamann, 2003; Rhoden and Bolek, 2012, 2015). Additionally, only Rhoden and Bolek (2015) examined tadpole stages of two anuran species from the same location for their helminth community structure. Consequently, little information is available about how tadpole species-specific differences in size, feeding strategy, and habitat and

how different transmission strategies of parasites play a role in structuring parasite communities of larval stages of anurans.

The amphibians of Oklahoma are comprised of 11 families and 58 species, with varied foraging strategies and habitats of larval stages of these amphibians (Sievert and Sievert, 2011; Altig and McDiarmid, 2015). There are also many sympatric species, therefore making Oklahoma an ideal location to examine tadpole stages of multiple species of anurans for their parasite community structure. However, currently limited information is available on amphibian parasites from the Southern Great Plains, including Oklahoma (Dyer and Brandon, 1973; Tyler and Buscher, 1980; McAllister et al., 1995; Vhora and Bolek, 2013, 2015), and no information is available on the parasites of tadpole stages of these anurans from Oklahoma. Thus, the present study aims to address these issues. Due to species-specific differences in diet, habitat, and physiology of various anurans species, I hypothesize that parasite communities of tadpole stages of anurans would differ significantly from those found in metamorphosed anurans. Since host factors such as species, and their habitat, size, and diet and/or feeding strategy, have previously been shown to affect parasite biodiversity and community structure within adult anurans, the present study addresses the following questions: 1) what species of parasites infect tadpole stages of five sympatrically occurring anuran species; 2) what is the parasite community structure at the infra-, component, and compound community levels within each anuran species and the tadpole community; and 3) does amphibian species, and tadpole size, habitat and/or their time to metamorphosis, play a role in determining parasite species and/or community distribution at the infra- and component community level? Finally, I evaluate the distribution and reproductive strategies of a tadpole specific

nematode, *Gyrinicola batrachiensis*, and their fungal symbionts infecting tadpole stages of five anuran species.

The present thesis is divided into two chapters including:

1) Parasite community structure in tadpole stages of five anuran species from north central Oklahoma.
2) Distribution and reproductive strategies of *Gyrinicola batrachiensis* (Oxyuroidea: Pharyngodonidae) and their fungal symbionts in tadpole stages of five species of anurans.

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CHAPTER II

TADPOLE PARASITE COMMUNITY STRUCTURE: DO PARASITE LIFE CYCLES MATTER?

ABSTRACT: Currently, little information is available on parasite community structure in larval amphibians, specifically larval anurans. I examined the parasite community structure in tadpoles of five anuran species from an ephemeral wetland in northcentral Oklahoma. Specifically, I was interested in how species-specific factors, such as size, feeding strategies, and habitat partitioning among larval anurans affect parasite community structure. Additionally, I was interested in how parasite life cycle strategies would affect their community composition. During May–August 2015 and April–June 2016, I collected tadpoles of southern leopard frogs, *Rana sphenocephala*, Blanchard's cricket frogs, *Acris blanchardi*, Cope's gray treefrogs, *Hyla chrysoscelis*, spotted chorus frogs, *Pseudacris clarkii*, and Great Plains narrow-mouthed toads, *Gastrophryne olivacea*. The compound parasite community was dominated by larval trematode stages (mesocercariae and metacercariae), with only two gravid adult helminth species present, the trematode *Megalodiscus temperatus*, and nematode *Gyrinicola batrachiensis*. The parasite component communities were depauperate in nature, with a maximum of six parasite species/types per component community. Although parasite host specificity

cannot be ruled out, my data indicate that tadpole size was the primary factor determining parasite abundances and intensities. However, after controlling for species-specific differences in tadpole size, parasite life cycle strategy and host species were the major factors affecting tadpole parasite community structure.

INTRODUCTION

In North America, anuran helminth community structure has been relatively well studied (Muzzall, 1991; Muzzall and Peebles, 1991; Yoder and Coggins, 1996; Bolek and Coggins 1998, 2000, 2001; Gillilland and Muzzall, 1999; Muzzall et al., 2001). Interspecific variation in diet, life histories, and body sizes among sympatric species of frogs and toads offer an excellent comparative approach for studies on helminth community structure (Aho, 1990; Goldberg et al., 1996; McAlpine, 1997; Bolek and Coggins, 2003; Yoder and Coggins, 2007). Additional studies on anuran helminth distributions and life cycles indicate that amphibian size, diet, and habitat and specific transmission strategies of helminths in these habitats are important factors in structuring anuran helminth communities (Bolek and Coggins, 2003; Bolek and Janovy, 2007a, b, 2008; Bolek et al., 2009, 2010; Muzzall and Peebles, 1991; Snyder and Janovy, 1994, 1996; McAlpine, 1997; Yoder and Coggins, 1996, 2007; Stigge and Bolek, 2015). On the aquatic–terrestrial anuran spectrum, parasite communities of aquatic species tend to have proportionately more aquatic digenetic trematode infections whereas terrestrial species have more directly-transmitted nematodes acquired from the soil (Bolek and Coggins, 2000, 2001; Yoder and Coggins, 2007). However, current knowledge of anuran parasite community structure is based primarily on surveys of adults which can vary dramatically on the aquatic–terrestrial habitat-use spectrum. In contrast, most tadpoles and larval

anurans are strictly aquatic, regardless of adult habitat-use patterns, and their parasites are not well understood (Rhoden and Bolek, 2012, 2015).

Tadpoles differ significantly from metamorphosed anurans in their biology (McDiarmid and Altig, 1999). Temperate-zone anuran tadpoles are found in aquatic habitats, where they feed on suspended and/or epibenthic algae and incidentally on zooplankton (Seale, 1980). The herbivorous lifestyle of aquatic tadpoles leads to a much larger intestine compared to those found in carnivorous anurans (McDiarmid and Altig, 1999). As a result, tadpole digestive systems and body morphology are dramatically different from those of the predatory adult anurans. Therefore, tadpoles have been considered vertebrate analogs to larvae of holometabolous insects (McDiarmid and Altig, 1999; Altig and McDiarmid, 2015). Despite numerous studies on ecological differences among tadpole stages of sympatrically distributed anuran species (Wilbur, 1972, 1976, 1980; Wilbur and Collins, 1973; Heyer, 1976; McDiarmid and Altig, 1999; Seale and Beckvar, 1980), only three studies have examined helminth community structure in tadpole stages of anurans (Kehr and Hamann, 2003; Rhoden and Bolek, 2012, 2015). Consequently, we know very little about how species-specific differences in tadpole size, feeding strategy, and habitat and specific transmission strategies of helminths play a role in structuring helminth communities in tadpoles of frogs and toads.

The goal of this study was to determine the factors that drive helminth communities in anuran tadpoles. I examined the parasite community structure of tadpoles of five sympatric and syntopic anuran species in northcentral Oklahoma and examined the effects of host and parasite characteristics on community structure. Specifically, I hypothesized species-specific differences in tadpole size, feeding strategies and habitat-

use would affect parasite community structure. Additionally, I hypothesized that parasite life cycle strategies would affect their community composition.

MATERIALS AND METHODS

Description of field site and anuran surveys

Teal Ridge, located in Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405), is 9.31 hectares of non-irrigated restored semi-permanent wetland containing four distinct ponds, which are surrounded by sedges (*Carex* spp.) and cattails (*Typha* spp.). These four ponds range in size from 183 m by 150 m to 100 m by 40 m and they are each less than 1 m deep. The largest pond is surrounded by the three smaller ponds, which are located 33 m to the north and east side of the largest pond. During the study, water was continuously present in the smallest pond from April of 2015–July of 2016; however, all tadpoles were collected with a dip-net from this pond and its overflow during May–August 2015 and April–June 2016. Tadpoles of at least two amphibian species were collected during each month and year of the study, and included southern leopard frogs, *Rana sphenocephala* (Family: Ranidae), Cope's gray treefrogs, *Hyla chrysocelis*, Blanchard's cricket frogs, *Acris blanchardi*, spotted chorus frogs, *Pseudacris clarkii* (Family: Hylidae), and Great Plains narrow-mouthed toads, *Gastrophryne olivacea* (Family: Microhylidae). However, tadpoles of *G. olivacea* were only collected during July 2015.

Tadpoles were transported to the laboratory in 19 L buckets filled with pond water without snails and euthanized with MS-222 (tricaine methanesulfonate) within 72 hours of capture. All tadpoles were identified using keys by Altig et al. (2010) and Altig

and McDiarmid (2015). Snout-vent length (SVL), total length (TL), tail height (TH) and Gosner stage (GS), or tadpole developmental stage, was recorded for tadpoles according to Gosner (1960) and McDiarmid and Altig (1999).

Because variances were heteroscedastic, the Kruskal–Wallis and the Dunn's multiple comparison tests were used to compare differences in SVL, TL and TH among tadpoles of different amphibian species. Additionally, the Kruskal–Wallis test and the Dunn's multiple comparison tests were used to compare differences in GS rank among tadpoles of different anuran species (Sokal and Rohlf, 1981). All tissues were examined for helminths and ectoparasitic crustaceans, including the skin, buccal cavity, gills, digestive tract, body musculature, internal organs, limbs, and tail. Each tadpole was visually observed for any parasites on the skin under a dissecting microscope and during necropsy each organ was placed in an individual Petri dish and examined with an Olympus SZ61 stereomicroscope. The kidneys were removed with forceps from the tadpole carcass and then pressed between two slides. Once kidney tissue was flattened, a wet mount was prepared by removing the top slide and adding a drop of water and covering the flattened tissue with a cover-slip. Slides were then examined with an Olympus BX-51 upright research microscope configured for bright field and differential interference contrast microscopy with plain fluorite objective for parasites. Finally, the entire musculature of each tadpole was teased apart with forceps in order to examine for encysted helminths and all worms were removed.

Adult trematodes were relaxed in tap water, heat killed and fixed in alcohol–formaldehyde–acetic acid or 95% ethanol. Nematodes were fixed in 70% ethanol. Trematode mesocercaria and metacercaria stages were examined live as wet mounts

using an Olympus BX-51 as previously described. Digital photographs were taken with an Olympus 5-megapixel digital camera before all mesocercariae and metacercariae stages were fixed in 70% or 95% ethanol. Adult trematodes and representative mesocercariae and metacercariae were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam, and all nematodes were cleared in glycerol and identified as temporary mounts (Pritchard and Kruse, 1982).

Parasite identification and community structure

Nematodes were identified based on the original descriptions in Adamson (1981a, 1981b) and general descriptions in Baker (1980). Adult trematodes were identified based on comparisons to previous descriptions in Krull and Price (1932) and Bolek and Janovy (2008); ectoparasitic crustaceans were identified based on Hoffman (1999); whereas larval trematodes were identified based on the descriptions in Prudhoe and Bray (1982) and Schell (1985).

Prevalence, mean intensity (MI), and mean abundance (MA) were calculated according to Bush et al. (1997). Mean parasite species richness (MSR) is the sum of parasite species and/or metacercarial types which could not be identified to species per individual anuran, including uninfected individuals, divided by the total sample size (Bolek and Coggins, 2003). Because variances were heteroscedastic, the Kruskal–Wallis test and the Dunn's multiple comparison tests were was used to compare differences in MA and MI for individual helminth species, and for overall helminth MA and MI, and MSR among different anuran species. An unequal variance student's *t*-test was used to compare differences in MA, MI and MSR when less than three species of anurans were infected with a particular helminth species. Finally, Pearson's correlation coefficient was

used to determine relationships among tadpole SVL, TL, TH and intensity and abundance for overall parasite intensities and abundances and parasite species richness. Spearman's rank correlation was used to determine these relationships with tadpole GS rank. The Šidák correction was used to correct for the number of correlations conducted. All values except prevalence are reported as the mean ± 1 SD (Sokal and Rohlf, 1981).

The role of parasite life cycles and host species in community structure

For additional analyses and to evaluate if anuran species-specific differences and/or parasite transmission strategies played a significant role in parasite community structure, parasite species were grouped based on transmission strategy, including those that infect tadpoles through skin contact or those that infect tadpoles through incidental ingestion. The ectoparasitic crustacean only infected a single tadpole and this parasite was removed from the analyses.

Helminth species were assigned to transmission strategies (skin contact or ingestion) based on life history descriptions and reviews for helminths in Adamson (1981a, 1981b), Schell (1985), Anderson (2000), Bolek and Janovy (2008) and Vhora and Bolek (2013). Because amphibian tadpole species-specific differences in size may play a role in the recruitment of skin-penetrating and ingested parasite, Homogeneity of Slopes Analyses of Covariance (HG-ANCOVA) were used to evaluate if TL had a significant host species-specific effect on parasites acquired by skin-penetration or ingestion. If homogeneity of slopes was observed, a Separate-Slopes Analysis of Covariance (SS-ANCOVA) was used which allows for continuous variables (i.e., TL) to covary with response variables (i.e., infections) within factors (i.e., amphibian species) followed by Fisher LSD post hoc tests. However, because MA and MI variances for skin

penetrating and incidentally ingested parasites were heteroscedastic, the Kruskal–Wallis test and the Dunn's multiple comparison tests were then used to evaluate significant differences on the predicted MA and MI values for skin penetrating and incidentally ingested helminths after anuran species TL was accounted for by SS-ANCOVAs. Data on predicted MA and MI values for skin penetrating and incidentally ingested helminths are presented as boxplots with a median, 25–75% interquartiles, and the range of lowest and highest predicted intensity and abundance values. Statistics were conducted using STATISTICA v10 (StatSoft, Inc., 2011).

Parasite community/aggregation analyses

Finally, to visualize tadpole parasite community structure and assess the relationships of parasite transmission strategies and amphibian host species with parasite communities in tadpoles, a partial redundancy analysis (RDA), was implemented in CANOCO 5 (Lepš and Šmilauer, 2003), factoring out tadpole size (TL). Further, we used a constrained RDA to test (999 permutations) the effect of host species on the composition of tadpole parasite communities, again factoring out tadpole size.

RESULTS

Anuran surveys

A total of 162 tadpoles of five anuran species were collected, and included 32 *R. sphenocephala*, 25 *H. chrysoscelis*, 72 *A. blanchardi*, 26 *P. clarkii*, and 7 *G. olivacea* (Fig. 1). Tadpoles of *R. sphenocephala* had the smallest GS rank, and longest mean SVL and longest mean TL. Tadpoles of *G. olivacea* had the shortest mean SVL and tadpoles of *P. clarkii* had the shortest mean TL (Table I). Finally, tadpoles of *H. chrysoscelis* had

the highest mean TH; whereas tadpoles of *P. clarkii* had the shortest mean TH (Table I). The Kruskal–Wallis test indicated that significant differences in GS, SVL, TL, and TH existed among the five species of anurans (Table I). The Dunn's multiple comparison tests indicated that *R. sphenocephala* was the only species with a significantly smaller GS rank ($P < 0.02$) than all other combinations of tadpoles; whereas all other treatment groups were not significantly different in GS rank ($P > 0.05$; Table I). Additionally, the Dunn's multiple comparison tests indicated that *R. sphenocephala* had a significantly greater SVL than *A. blanchardi, P. clarkii* and *G. olivacea* and *H. chrysoscelis* had a significantly greater SVL than *P. clarkii* and *G. olivacea*; whereas all other treatment groups were not significantly different in average SVL ($P < 0.01$ for all significant differences; Table I). Similar relationships were observed for TL ($P < 0.05$ for all significant differences) with the exception that there was no significant difference in TL for *R. sphenocephala*, and *H. chrysoscelis* (Table I). Finally, the Dunn's multiple comparison tests revealed that TH of *H. chrysoscelis* and *R. sphenocephala* was not significantly different between the two species ($P > 0.05$) and there were no significant differences in TH among *A. blanchardi, P. clarkii* and *G. olivacea* ($P > 0.05$; Table I). However, both *H. chrysoscelis* and *R. sphenocephala* had a significantly greater TH than *A. blanchardi, P. clarkii* and *G. olivacea* ($P < 0.008$ for all significant differences; Table I).

Parasite diversity and distribution in tadpoles

The parasite compound community of these five species of larval anurans consisted of at least ten species of helminths and ectoparasitic crustaceans. These included a single species of adult gravid trematode (*Megalodiscus temperatus*), one

species of adult gravid nematode (*Gyrinicola batrachiensis*), two species of immature nematodes (*Aplectana* sp. and an unidentified nematode), five species/types of mesocercariae and metacercariae trematode stages (Fig. 2), and a single species of an immature ectoparasitic crustacean (*Lernaea* sp.; Table II). Prevalence was highest (80%) for *G. batrachiensis* in *A. blanchardi*; whereas MA (20.03 ± 50.32) and MI (45.79 ± 69.05) were highest for mesocercariae stages of *Fibricola* sp. in *R. sphenocephala* (Table II). Of those, *Aplectana* sp. and *Lernaea* sp. are accidental parasites of tadpoles and commonly infect metamorphosed anurans or fish, respectively (see Hoffman, 1999; Vhora and Bolek, 2013).

Of the 3,132 parasites recovered, 67 % (2,107) of the compound community was dominated by larval trematode stages (mesocercariae and metacercariae) acquired by active penetration in the water column, followed by an adult trematode species (*M. temperatus*; 19.6 %; 613) and three species of nematodes (*G. batrachiensis*, *Aplectana* sp. and an unidentified nematode; 13.1%; 411) acquired by incidental ingestion of metacercariae, eggs, or larval stages of these parasites by tadpoles on the pond bottom, respectively. Overall prevalence, MA, MI and MSR were generally higher in tadpoles of *R. sphenocephala*, *H. chrysoscelis*, and *A. blanchardi*, and lower in tadpoles of *P. clarkii* and *G. olivacea* (Table III). The Kruskal–Wallis tests showed that significant differences in overall MA, MI, and MSR existed among tadpoles of the five species of anurans (Table III). For overall MA and MSR, the Dunn's multiple comparison tests indicated there were no significant differences ($P > 0.05$) in overall MA and MSR between tadpoles of *R. sphenocephala*, *A. blanchardi* and *H. chrysoscelis*, or *P. clarkii* and *G. olivacea*. However, significant differences existed ($P < 0.01$ for all significant

comparisons) for overall MA and MSR among the three anurans species with large tadpoles (*R. sphenocephala*, *A. blanchardi* and *H. chrysoscelis*) and two anuran species with small tadpoles (*P. clarkii* and *G. olivacea*; Table III). For overall MI, the Dunn's multiple comparison tests indicated that *R. sphenocephala* had a significantly higher MI than *P. clarkii* and *G. olivacea* ($P < 0.005$); whereas all other treatment groups were not significantly different for MI ($P > 0.05$; Table III). Finally, significant and relatively strong positive correlations existed for SVL, TL, TH and GS and overall MA or MSR for *R. sphenocephala*; SVL, TL, TH and overall MA and/or MSR for *A. blanchardi*; and SVL, TL and TH and MA and MSR for total sample of tadpoles collected; whereas all other correlations for tadpoles of the remaining amphibian species were not significant ($P > 0.05$; Table IV).

The role of parasite life cycles and host species in community structure

The HG-ANCOVA indicated that TL has significant host species-specific effects on skin-penetrating parasite abundance ($F_{4, 152} = 7.44$; $P < 0.0001$) and intensity ($F_{4, 105} = 4.29$; $P < 0.003$). The SS-ANCOVAs indicated significant host species-specific size effects on skin-penetrating parasite abundance ($F_{5, 152} = 14.24$; $P < 0.0001$) and intensity ($F_{5, 105} = 8.75$; $P < 0.00001$; Table V). After accounting for host-specific size–infection relationships, skin-penetrating parasite abundance ($F_{4, 152} = 5.29$; $P < 0.0005$) and intensity ($F_{4, 105} = 3.06$; $P < 0.02$) were significantly different among host species, such that *R. sphenocephala* had a significantly greater skin-penetrating parasite abundance (Post Hoc Fisher LSD < 0.05) and intensity (Post Hoc Fisher LSD < 0.05) than all other amphibian species.

After accounting for amphibian species-specific difference in TL, the Kruskal–Wallis tests indicated that significant differences existed for predicted MA ($H_{4, 162}$ = 75.78; P = 0.0001) and predicted MI ($H_{4, 115}$ = 56.06; P = 0.0001) for skin penetrating helminths among the five species of anurans. The Dunn's multiple comparison tests indicated there were no significant differences in predicted MA for skin penetrating helminths for *R. sphenocephala* and *H. chrysoscelis* or *P. clarkii* and *G. olivacea* (P > 0.05). In contrast, *R. sphenocephala* and *H. chrysoscelis* had significantly higher predicted MA of skin penetrating parasites than *A. blanchardi*, *P. clarkii* and *G. olivacea*; whereas *A. blanchardi* was significantly different from all groups, having a lower predicted MA than *R. sphenocephala* and *H. chrysoscelis* and significantly higher predicted MA than *P. clarkii* and *G. olivacea* (Fig. 3). For predicted MI the Dunn's multiple comparison tests indicated that there were no significant differences for *R. sphenocephala* and *H. chrysoscelis* or *A. blanchardi*, *P. clarkii* and *G. olivacea* (P > 0.05); whereas *R. sphenocephala* and *H. chrysoscelis* had significantly higher predicted MI than *A. blanchardi*, *P. clarkii* and *G. olivacea* (P < 0.002 for all comparisons; Fig. 3).

For ingestion-mode parasites, *G. olivacea* was removed from the analysis because this species did not have any of these infections. The HG-ANCOVA indicated TL had significant host species-specific effects on ingestion-mode parasite abundance ($F_{3, 147}$ = 3.87; P = 0.011) but not on ingestion-mode parasite intensity ($F_{3, 92}$ = 1.18; P = 0.32). Intensity of ingestion-mode parasites was the only case where TL remained significant ($F_{1, 92}$ = 6.01; P = 0.016) after accounting for host species-specific TL-parasite relationships.

SS-ANCOVAs also indicated significant host species-specific size–infection relationships for ingestion-mode parasite abundance ($F_{4, 147} = 18.30$, $P < 0.001$) and intensity ($F_{4, 92} = 6.86$, $P < 0.001$; Table V). After accounting for host species-specific size effects, ingestion mode parasite abundance was significantly different among anuran species ($F_{3, 147} = 3.58$; $P = 0.015$) whereas intensity was not ($F_{3, 92} = 0.85$; $P = 0.47$). *Acris blanchardi* and *R. sphenocephala* had significantly greater MA for ingestion mode parasites than *H. chrysoscelis* (Post Hoc Fisher LSD < 0.05) and *A. blanchardi* had significantly greater MI for ingestion mode parasites than *H. chrysoscelis* and *P. clarkii*; whereas *R. sphenocephala* had significantly greater MI for ingestion mode parasites than *P. clarkii* (Post Hoc Fisher LSD < 0.05).

Once size was accounted for, the Kruskal–Wallis tests indicated that significant differences existed for predicted MA ($H_{3, 155} < 64.93$; $P = 0.0001$) and predicted MI ($H_{3, 100} < 32.02$; $P = 0.0001$) for ingested-mode parasites among the four anuran species. The Dunn's multiple comparison tests indicated there were no significant differences in predicted MA for ingested helminths for *R. sphenocephala, A. blanchardi* or *H. chrysoscelis* ($P > 0.05$). In contrast, *A. blanchardi* had significantly higher predicted MA of ingested helminths than *H. chrysoscelis* ($P < 0.00001$) and *R. sphenocephala, A. blanchardi* and *H. chrysoscelis* had significantly higher predicted MA of ingested helminths than *P. clarkii* ($P < 0.0004$ for all significant groups; Fig. 4). For predicted MI of ingested-mode parasites the Dunn's multiple comparison tests indicated that there were no significant differences for *R. sphenocephala* and *A. blanchardi*, or *H. chrysoscelis* and *P. clarkii* ($P > 0.05$). However, *R. sphenocephala* and *A. blanchardi* had

significantly higher predicted MI than *H. chrysoscelis* and *P. clarkii* ($P < 0.002$ for all comparisons; Fig. 4).

Parasite community/aggregation analyses

The microcrustacean only infected a single *A. blanchardi* and thus was removed from the multivariate analyses. A partially-constrained RDA (tadpole size factored out) indicated strong aggregation among the ingestion-mode parasites (*Aplectana* sp., *M. temperatus*, and *G. batrachiensis*) and among skin-penetrating parasites (*Alaria* sp., *Fibricola*, sp., type 1, type 2 and echinostomatid metacercariae) but separation among the two guilds. Unconstrained RDA axis 1 accounted for the most variation in parasite community/aggregate structure and separated the parasite community primarily based on mode of transmission (ingestion vs. penetration; Fig. 5). Constraining the analysis to host species (still factoring host size out) did not improve model fit and only accounted for 4.3% of the total variation compared to 72.6% in the unconstrained ordination. However, and although only describing a small portion of the variance, host species did have a significant effect on community/aggregate structure (*pseudo-F* = 1.8; *P* = 0.042). Ingestion-mode parasites (*M. temperatus* and *G. batrachiensis*) were common and abundant in *A. blanchardi*; whereas *G. olivacea* and *P. clarkii* had similar parasite aggregations, with high abundances of skin-penetrating parasites. *Rana sphenocephala* had a combination of skin-penetrating and ingestion-mode parasites and was the primary host for *Alaria* sp., which made it cluster differently from the other species. Finally, *H. chrysoscelis* was infected by nearly every species except *Aplectana* sp. and thus did not have any associations with group of parasites with skin-penetrating or ingestion-mode parasites (Fig. 5).

DISCUSSION

A major contribution of this study is that it is only the second study to examine parasite community structure in tadpoles of sympatrically occurring anurans. Additionally, my study indicates that helminth communities in tadpole stages of anurans are not as clearly defined as they are in metamorphosed anurans which have distinct ecological habitats (terrestrial, aquatic, arboreal, etc.). Although parasite host specificity cannot be ruled out, anuran species-specific differences in tadpole habitat partitioning (benthic or pelagic) and feeding strategies (filter feeders, bottom feeders or both), as well as distinct parasite transmission strategies (incidentally ingested or through skin contact), appeared to be important in structuring parasite communities at the component level in tadpole stages of these anuran species. Several distinct differences were evident at the component helminth community level among tadpoles of sympatric anurans. My data indicate that tadpole size is the primary factor determining parasite abundances and intensities. However, after controlling for species-specific differences in tadpole size, parasite life cycle strategy and host species were the major factors affecting tadpole parasite community structure.

Although tadpoles of these five anuran species all inhabit the same body of water, they are known to have differences in habitat preferences and/or feeding strategies within ponds. Tadpoles of *R. sphenocephala* and *A. blanchardi* are predominantly benthic in their habitat and have deep bodies with less developed tail musculature allowing them to forage on filamentous algae and surface detritus on the pond bottom, but can also feed on plankton in the open water column (Dodd, 2013; Altig and McDiarmid, 2015). Tadpoles

of *A. blanchardi* usually occur in 2–4 cm of water at the very margins of ponds among debris below emergent vegetation; whereas tadpoles of *R. sphenocephala* are found in deeper water but commonly enter shallow areas with submerged vegetation to bask (Dodd, 2013; Altig and McDiarmid, 2015). In contrast, tadpoles of *H. chrysoscelis* are nektonic (i.e., swim and migrate freely) and are commonly found in the open water column, but consume algae and detritus rasped from plant material and the substrate on the pond bottom (Heyer, 1976; Dodd, 2013). Although nothing is known about the ecology of tadpoles of *P. clarkii*, studies on tadpoles of other *Pseudacris* species indicate they swim at or near the surface of the water where they consume algae but may also feed on detritus on the pond bottom (Heyer, 1976; Dodd, 2013). Finally, tadpoles of the microhylid *G. olivacea* usually hide on the bottom during the day and suspension feed quiescently on phytoplankton or zooplankton at or near the surface at night (Dodd, 2013; Altig and McDiarmid, 2015).

As in previous studies on parasite community structure in amphibian hosts, host size was an important factor in structuring parasite communities of tadpoles in this study. Amphibian species with larger tadpoles had significantly higher MA, MI and/or MSR, and/or within species larger tadpoles were positively correlated with parasite abundance, intensity and species richness for some amphibian species (McAlpine, 1997; Bolek and Coggins, 2000, 2001, 2003; Yoder and Coggins, 2007; Campião et al., 2015). However, after accounting for amphibian species-specific difference in size, significant differences in abundance and intensities were observed for skin contact and ingested mode parasites among the five amphibian species.

For the ingestion mode parasites, tadpoles become infected with *M. temperatus*, *G. batrachiensis,* and *Aplectana* sp. while feeding on the pond bottom and incidentally ingesting the metacercaria, egg or larval stages of these helminths, respectively (Adamson, 1981a, b; Anderson, 2000; Bolek and Janovy, 2008). Therefore, finding significantly higher MA and/or MI of ingested mode parasites in tadpoles that are more benthic such as *R. sphenocephala* and *A. blanchardi* than *H. chrysoscelis* and *P. clarkii* strongly suggests that habitat partitioning and differences in feeding behavior among these anuran species provide avenues and constraints for exposure to parasites transmitted through incidental ingestion. Although the sample size for *G. olivacea* was low in this study, tadpoles of this anuran species spend a significant portion of their time on the pond bottom during the day and therefore overlap in their habitat with life cycle stages of the three ingested mode parasites. However, no tadpoles of this species were infected with any ingested mode parasites. Importantly, tadpoles of this anuran species are strictly filter feeders and their mouthparts are incapable of rasping algae or other surface detritus (Dodd, 2013; Altig and McDiarmid, 2015). This strongly suggests that the lack of any ingested mode parasites observed in *G. olivacea* is due to the inability of tadpoles of this species of anuran to ingest metacercaria, egg, or larval stages of these parasites.

For parasites with the skin-contact mode of transmission (*Alaria* sp., *Fibricola*, sp., type 1, type 2 and echinostomatid metacercariae), explanations for the observed differences in the distribution, abundance and intensities among tadpoles of the five anuran species are more difficult to interpret. In order for tadpoles to become infected with any of these trematode larval stages, cercarial stages are released from snail first

intermediate hosts into the water, and those cercariae must encounter and penetrate a suitable tadpole host within a short time frame, usually 24-48 hours (Schell, 1985). However, one major difference among the different trematodes infecting tadpoles in this study is the behavior of their cercariae (Schell, 1985).

For example, once released from their snail hosts, cercariae of the strigeoid trematodes *Alaria* sp. and *Fibricola* sp. hang in a resting position in the water column and slowly sink to the bottom of the pond (Schell, 1985). When tadpoles swim near the cercariae and create currents, the cercariae become active, attach, and penetrate tadpoles. However, cercariae of *Alaria* sp. are strongly phototrophic (i.e., respond to light) and are found in the upper water column; whereas cercariae of *Fibricola* sp. are not phototrophic and are distributed throughout the water column (Schell, 1985). This difference in cercariae behavior suggests one explanation of why tadpoles of some anuran species not found in the upper water column during the day such as *G. olivacea* or concealed among debris on the pond bottom such as *A. blanchardi* may not come in contact with the cercariae of *Alaria* sp. commonly (Schell, 1985; Dodd, 2013; Altig and McDiarmid, 2015).

Unfortunately, the other trematode larval stages infecting tadpoles in this study could not be identified to generic or species level and it is unclear what role host specificity, or host parasite encounter have on the distribution of these trematodes in different amphibian species in this system. Nevertheless, the significantly higher predicted MA and MI for skin-contact parasites in *R. sphenocephala* and *H. chrysoscelis* than the other three amphibian species suggest that these tadpoles have higher vagility than tadpoles of the other anuran species. For example, tadpoles of *R. sphenocephala*

commonly move from deep water to shallow water for basking; whereas tadpoles of *H. chrysoscelis* are considered nektonic and constantly move between the open water column and the pond bottom where they feed (Dodd, 2013). As a result, tadpoles of both of these anuran species may sample more of their environment and in the process increase their encounter rate with skin contact parasites. This hypothesis is supported by the fact that tadpoles of *R. sphenocephala* and *H. chrysoscelis* were the only amphibian species infected with all five skin contact helminths recovered in this study. Taken together, these observations strongly suggest that transmission strategies of amphibian parasites and potential host encounter with those parasites in specific environments were significant factors in structuring parasite communities in these amphibian hosts.

Although species-specific differences existed in the species/types of parasites infecting different anuran species, redundancy multivariate analysis indicated strong aggregation among the ingestion-mode parasites and among skin-contact parasites but these aggregations separated among the two modes of transmission. Surprisingly, incorporating host species did not improve model fit, strongly supporting the hypothesis that parasite life cycle transmission strategies were the other major drivers of parasite community structure in this system. However, although only explaining a small portion of the variance, host species did have a significant effect on parasite community/aggregate structure indicating that host habitat, parasite host specificity, or a combination of these factors played a role in structuring tadpole parasite communities in this system.

As in previous studies on helminth community structure in larval amphibians, the parasite communities in tadpole stages of this anuran assemblage were depauperate (Kehr

and Hamann, 2003; Rhoden and Bolek, 2012, 2015). In terms of species richness, prevalence, and abundance, the compound helminth community was dominated by larval (mesocercaria and metacercaria) stages of trematodes, with only a single species of adult nematode and adult trematode observed. These data support previous studies on helminth community structure of tadpoles where larval stages of trematodes were the dominant helminth groups of parasites infecting tadpole stages of anurans (Kehr and Hamann 2003; Rhoden and Bolek 2012, 2015)

Clearly, tadpoles of anurans are extremely diverse in their biology, habitat, and life histories, as are the adult anurans of these stages. However, in order for us to get a better understanding of the important factors structuring helminth communities of anuran tadpoles, more work needs to be done in these systems. I hope my work stimulates studies in this area.

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Table I. Developmental time (DT*) in weeks; average snout-vent length (SVL), total length (TL) and tail height (TH) in mm ± 1SD (range); range of Gosner stages (GS) and values for five species of anuran tadpoles collected from Teal Ridge, Stillwater, Oklahoma. Lowercase letters represent significant differences in mean abundance among host combinations ($P \leq 0.03$ for all significant differences).

	R. sphenocephala (n=32)	*A. blanchardi* (n=72)	*H. chrysoscelis* (n=25)	*P. clarkii* (n=26)	*G. olivacea* (n=7)	Statistic	*P*<
DT	8.5-12.5	4.1-12.9	3.4-9.3	4-6	2.9-10		
SVL	17.92 ± 4.92[a] (12-29)	13.74 ± 2.36[b] (7-19)	15.08 ± 1.90[ab] (11-18)	10.62 ± 2.30[c] (7-18)	10.07 ± 0.45[c] (9.5-11)	H = 66.16	**0.0001**
TL	40.47 ± 14.05[a] (21.5-71.5)	32.45 ± 6.13[a] (16.5-44)	35.00 ± 5.39[a] (25-46.5)	24.69 ± 7.77[b] (13-46)	25.21 ± 2.14[b] (21-28)	H = 40.72	**0.0001**
TH	8.92 ± 3.36[a] (18.5-4.5)	5.99 ± 1.57[a] (3-9.5)	9.18 ± 1.63[b] (5.5-12)	5.29 ± 1.83[b] (2-10)	5.36 ± 0.38[b] (5-6)	H = 64.04	**0.0001**
GS	26 – 44[a]	26 – 46[b]	30 – 42[b]	29 – 45[b]	36 – 39[b]	H = 32.59	**0.0001**

*Data referenced in Altig and McDiarmid (2015).

Table II. Prevalence (%; No. infected/No. examined) and 95% confidence intervals, mean intensity (MI), and mean abundance (MA) values for helminths and crustaceans recovered from tadpoles of five anuran species collected from Teal Ridge, Stillwater, Oklahoma. Lowercase letters represent significant differences in mean abundance among host combinations ($P < 0.05$ for all significant differences).

Species	Measure of Parasitism	*R. sphenocephala* (n=32)	*A. blanchardi* (n=72)	*H. chrysoscelis* (n=25)	*P. clarkii* (n=26)	*G. olivacea* (n=7)	Location*	Statistic	*P*
Trematoda									
†*Megalodiscus temperatus*	% 95% CI	41 (13/32) 25 – 58	47 (34/72) 36 – 59	28 (7/25) 14 – 48	12 (3/26) 3 – 30	0 (0/7) 0 – 40	LI		
	MA ± 1SD	5.5 ± 9.77^{ab}	5.67 ± 8.79^{a}	1.04 ± 2.3^{ab}	0.12 ± 0.33^{b}	0		H = 15.689	**P = 0.0013**
	MI ± 1SD (range)	13.54 ± 11.34^{a} (1-39)	12 ± 9.39^{a} (2-36)	3.71 ± 3.09^{ab} (1-9)	1 ± 0.0^{b} (1)	-		H = 14.884	**P = 0.0019**
Alaria sp.	% 95% CI	16 (5/32) 6 – 32	0 (0/72) 0 – 6	4 (1/25) 0.5 – 21	0 (0/26) 0 – 15	0 (0/7) 0 – 40	BC		
	MA ± 1SD	1.78 ± 6.73^{a}	0	0.08 ± 0.4^{a}	0	0		t = -1.259	P = 0.2133
	MI ± 1SD (range)	11.4 ± 14.64 (2-37)	-	2 (2)	-	-		N/A	N/A
Fibricola sp.	% 95% CI	44 (14/32) 28 – 61	11 (8/72) 5 – 21	60 (15/25) 41 – 77	58 (15/26) 39 – 74	14 (1/7) 0.5 – 53	BC, BM		
	MA ± 1SD	20.03 ± 50.32^{a}	1.04 ± 7.54^{b}	8.72 ± 15.18^{a}	2.73 ± 5.67^{a}	0.29 ± 0.76^{ab}		H = 34.746	**P < 0.0001**
	MI ± 1SD (range)	45.79 ± 69.05^{ac} (1-252)	9.25 ± 22.13^{b} (1-64)	14.53 ± 17.45^{c} (1-66)	4.73 ± 6.87^{b} (1-26)	2^{abc} (2)		H = 18.068	**P = 0.0012**
Echinostomatid metacercaria	% 95% CI	3 (1/32) 0.5 – 17	1 (1/72) 0.5 – 8	4 (1/25) 0.5 – 21	0 (0/26) 0 – 15	0 (0/7) 0 – 40	K		

	MA ± 1SD	0.031 ± 0.18	0.014 ± 0.12	0.32 ± 1.6	0	0		*H* = 1.641	*P* = 0.8013
	MI ± 1SD (range)	1 (1)	1 (1)	8 (8)	-	-		N/A	N/A
Type 1 metacercaria	% 95% CI	72 (23/32) 54 – 85	61 (44/72) 50 – 72	72 (18/25) 52 – 86	38 (10/26) 22 – 58	57 (4/7) 25 – 84	BC, BM, K		
	MA ± 1SD	14.59 ± 39.41[a]	2.97 ± 4.29[ab]	4.12 ± 7.01[ab]	0.77 ± 1.39[b]	0.71 ± 0.76[ab]		*H* = 16.050	***P* = 0.0030**
	MI ± 1SD (range)	20.30 ± 45.47[a] (1-217)	4.76 ± 4.58[ab] (1-24)	5.72 ± 7.72[ab] (1-28)	2 ± 1.63[b] (1-6)	1.25 ± 0.5[b] (1-2)		*H* = 15.756	***P* = 0.0034**
Type 2 metacercaria	% 95% CI	34 (11/32) 20 – 52	15 (11/72) 9 – 26	32 (8/25) 17 – 52	0 (0/26) 0 – 15	0 (0/7) 0 – 40	BC, BM, K		
	MA ± 1SD	4.75 ± 13.37[a]	0.71 ± 2.91[b]	0.76 ± 1.27[b]	0	0		*H* = 6.048	***P* = 0.0486**
	MI ± 1SD (range)	13.82 ± 20.41 (1-68)	4.64 ± 6.34 (1-23)	2.38 ± 1.06 (1-4)	-	-		*H* = 1.765	*P* = 0.4138
Nematoda †*Gyrinicola batrachiensis*	% 95% CI	41 (13/32) 25 – 58	83 (60/72) 73 – 90	52 (13/25) 34 – 70	15 (4/26) 5 – 34	0 (0/7) 0 – 40	LI		
	MA ± 1SD	3.69 ± 8.04[a]	3.19 ± 3.94[b]	1.68 ± 2.88[b]	0.73 ± 3.13[ab]	0		*H* = 32.413	***P* = 0.004**
	MI ± 1SD (range)	9.08 ± 10.68 (1-36)	3.83 ± 4.03 (1-21)	3.23 ± 3.35 (1-10)	4.75 ± 7.5 (1-16)	-		*H* = 7.208	*P* = 0.0656
Aplectana sp.	% 95% CI	3 (1/32) 0.5 – 17	0 (0/72) 0 – 6	0 (0/25) 0 – 16	0 (0/26) 0 – 15	0 (0/7) 0 – 40	LI		
	MA ± 1SD	0.031 ± 0.18	0	0	0	0		N/A	N/A

	MI ± 1SD (range)	1 (1)	-	-	-	-		N/A	N/A
Unidentified nematode	% 95% CI	0 (0/32) 0 – 13	1 (1/72) 0.5 – 8	0 (0/25) 0 – 16	0 (0/26) 0 – 15	0 (0/7) 0 – 40	BM		
	MA ± 1SD	0	0.014 ± 0.12	0	0	0		N/A	N/A
	MI ± 1SD (range)	-	1 (1)	-	-	-		N/A	N/A
Arthropoda *Lernaea* sp.	% 95% CI	0 (0/32) 0 – 13	1 (1/72) 0.5 – 8	0 (0/25) 0 – 16	0 (0/26) 0 – 15	0 (0/7) 0 – 40	BS		
	MA ± 1SD	0	0.014 ± 0.12	0	0	0		N/A	N/A
	MI ± 1SD (range)	-	1 (1)	-	-	-		N/A	N/A

*BC = body cavity, BM = body musculature, BS = body surface, K = kidneys, LI = large intestine. †Gravid helminths.

Table III. Overall helminth prevalence (%; No. infected/No. examined), overall mean abundance (MA), overall mean intensity (MI), and mean species richness (MSR) for helminth parasites recovered from tadpoles of five species of anurans collected from Teal Ridge, Stillwater, Oklahoma. Lowercase letters represent significant differences in mean abundance among host combinations ($P \leq 0.03$ for all significant differences).

	R. sphenocephala (n=32)	*A. blanchardi* (n=72)	*H. chrysoscelis* (n=25)	*P. clarkii* (n=26)	*G. olivacea* (n=7)	Statistic	*P*
% 95% CI	81 (26/32) 64 – 91	99 (71/72) 92 – 99	88 (22/25) 69 – 97	69 (18/26) 50 – 84	57 (4/7) 25 – 84		
MA ± 1SD (range)	50.41 ± 91.96[a] (0–388)	13.68 ± 16.05[a] (0–104)	16.72 ± 20.61[a] (0–91)	4.35 ± 6.72[b] (0–28)	1 ± 1.15[b] (0–3)	$H = 28.671$	$\mathbf{P < 0.0001}$
MI ± 1SD	62.08 ± 98.66[a]	13.82 ± 16.07[ab]	19.00 ± 20.98[ab]	6.33 ± 7.40[b]	1.75 ± 0.96[b]	$H = 22.76$	$\mathbf{P = 0.001}$
MSR ± 1SD (range)	2.5 ± 1.78[a] (0–6)	2.29 ± 1.24[a] (0–5)	2.5 ± 1.53[a] (0–6)	1.27 ± 1.15[b] (0–4)	0.71 ± 0.76[ab] (0–2)	$H = 21.032$	$\mathbf{P = 0.0003}$

Table IV. Pearson's correlations (r) for mean abundance (MA) and mean species richness (MSR) and Spearman's rank correlations (r_s) for Gosner stage (GS) and snout-vent length (SVL), total length (TL), and tail height (TH) for each amphibian species and total tadpoles collected from Teal Ridge, Stillwater, Oklahoma. Numbers in bold represent statistically significant r or r_s values ($P < 0.05$).

	R. sphenocephala (n=32)		*A. blanchardi* (n=72)		*H. chrysoscelis* (n=25)		*P. clarkii* (n=26)		*G. olivacea* (n=7)		Total (n=162)	
	MA	MSR	MA	MSR	MA	MSR	MA	MSR	MA	MSR	MA	MSR
SVL	**0.658**	**0.670**	**0.348**	0.150	0.266	0.193	0.255	0.456	0.321	0.070	**0.595**	**0.479**
TL	**0.674**	**0.651**	**0.307**	0.125	0.136	0.192	0.0209	0.316	-0.405	-0.471	**0.587**	**0.457**
TH	**0.643**	**0.579**	**0.419**	**0.424**	0.109	0.036	-0.116	0.199	0.382	0.125	**0.535**	**0.436**
GS	**0.535**	**0.520**	0.215	-0.067	0.230	0.141	0.167	0.128	0.093	-0.096	0.158	0.061

Table V. Overall mean abundance (MA), and overall mean intensity (MI), for skin contact and ingested type transmission of helminth parasites recovered from tadpoles of five species of anurans collected from Teal Ridge, Stillwater, Oklahoma.

	R. sphenocephala (n=32)	*A. blanchardi* (n=72)	*H. chrysoscelis* (n=25)	*P. clarkii* (n=26)	*G. olivacea* (n=7)	Statistic	*P*
Skin contact							
MA ± 1SD (range)	41.19 ± 87.30 (0–366)	4.75 ± 10.75 (0–77)	14.00 ± 19.28 (0–87)	3.50 ± 6.2 (0–27)	1 ± 1.15 (0–3)	$F = 14.24$	$\mathbf{P < 0.0001}$
MI ± 1SD	52.72 ± 95.97	6.98 ± 12.45	16.67 ± 19.98	5.69 ± 7.15	1.75 ± 0.96	$F = 8.75$	$\mathbf{P < 0.0001}$
Ingested							
MA ± 1SD (range)	9.22 ± 14.70 (0–48)	8.86 ± 10.04 (0–40)	2.72 ± 4.59 (0–19)	0.84 ± 3.13 (0–16)	0	$F = 18.30$	$\mathbf{P < 0.001}$
MI ± 1SD	15.52 ± 16.39	10.63 ± 10.10	4.25 ± 5.17	3.67 ± 6.05	0	$F = 6.68$	$\mathbf{P < 0.001}$

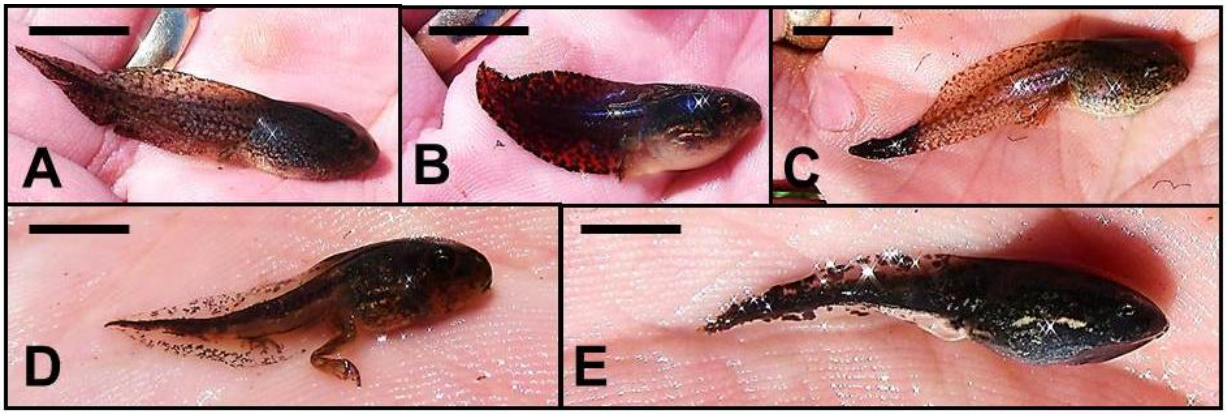

FIGURE 1. (**A**) *Rana sphenocephala*; Scale bar = 18 mm. (**B**) *Hyla chrysoscelis*; Scale bar = 15 mm. (**C**) *Acris blanchardi*; Scale bar = 14 mm. (**D**) *Pseudacris clarkii*; Scale bar = 11 mm. (**E**) *Gastrophryne olivacea*; Scale bar = 10 mm.

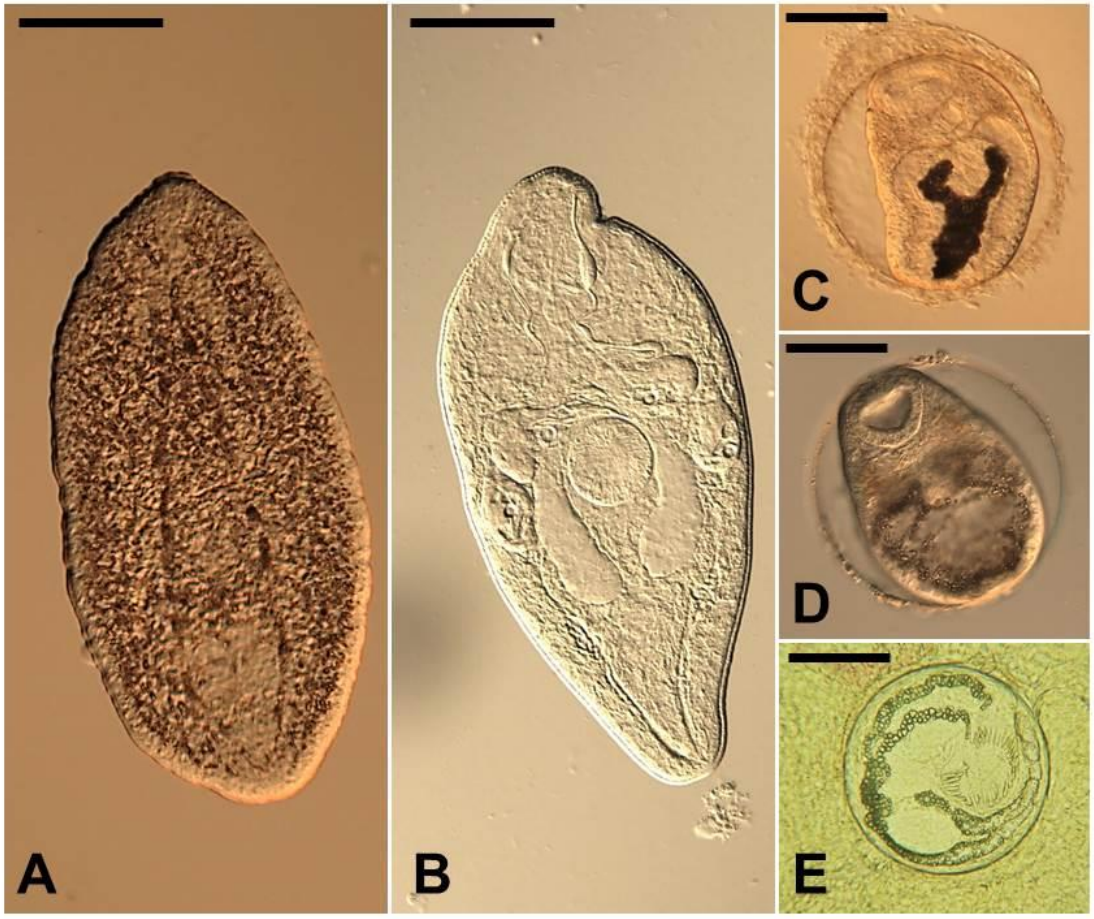

FIGURE 2. Larval stages of trematodes infecting tadpoles of 5 different anuran species collected at Teal Ridge. (**A**) Mesocercaria stage of *Fibricola* sp. from the body cavity of *R. sphenocephala*. (**B**) Mesocercaria stage of *Alaria* sp. from the body cavity of *R. sphenocephala*. (**C**) Type 1 metacercia from the musculature of a *G. olivacea*. Note the dark Y shaped excretory vesicle and long intestinal ceacae. (**D**) Type 2 metacercia from the musculature of an *A. blanchardi*. Note the short intestinal ceacea and gray Y shaped excretory vesicle with less distinct branches. (**E**) Echinostomatid metacercaria from the kidney of *H. chrysoscelis*. Note the granual excretory vesicle and distinct collar spines. All scale bars = 50 μm.

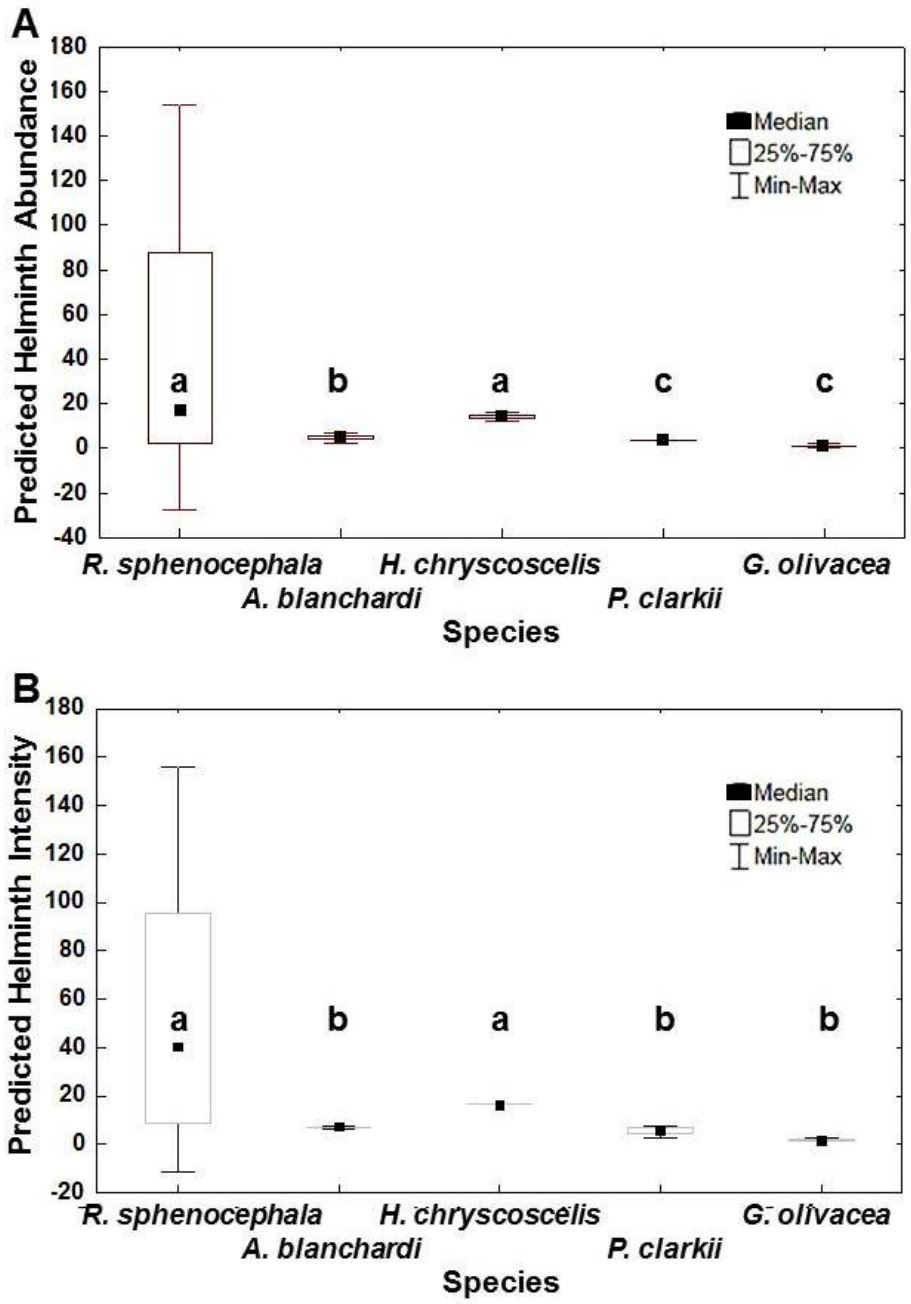

FIGURE 3. Boxplots representing medians, 25–75% interquartiles, and ranges for predicted mean abundance (**A**) and predicted mean intensity (**B**) for skin-contact parasites infecting tadpoles of five species of anurans collected from Teal Ridge, Stillwater OK.

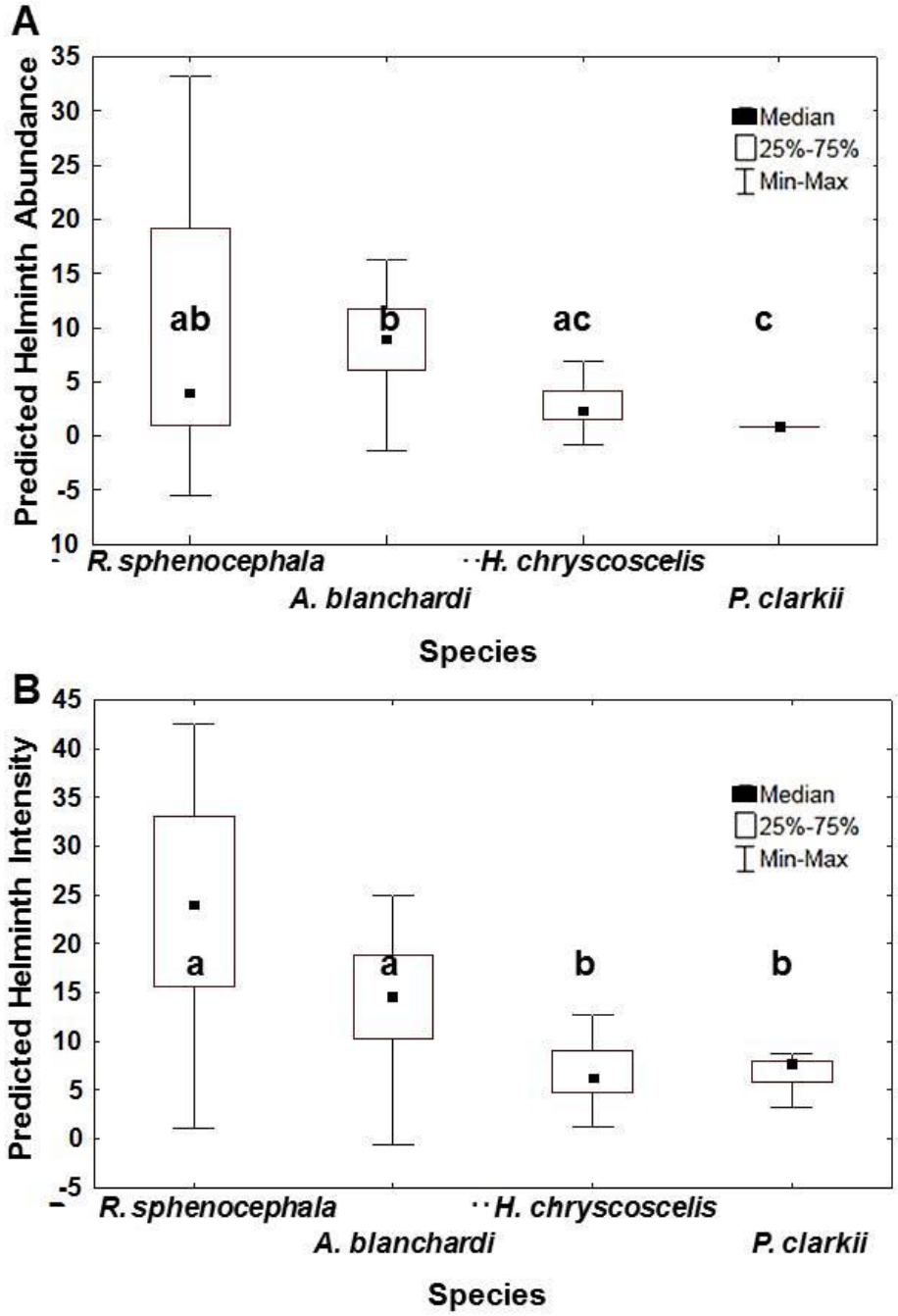

FIGURE 4. Boxplots representing medians, 25–75% interquartiles, and ranges for predicted mean abundance (**A**) and predicted mean intensity (**B**) for ingested parasites infecting tadpoles of four species of anurans collected from Teal Ridge, Stillwater OK.

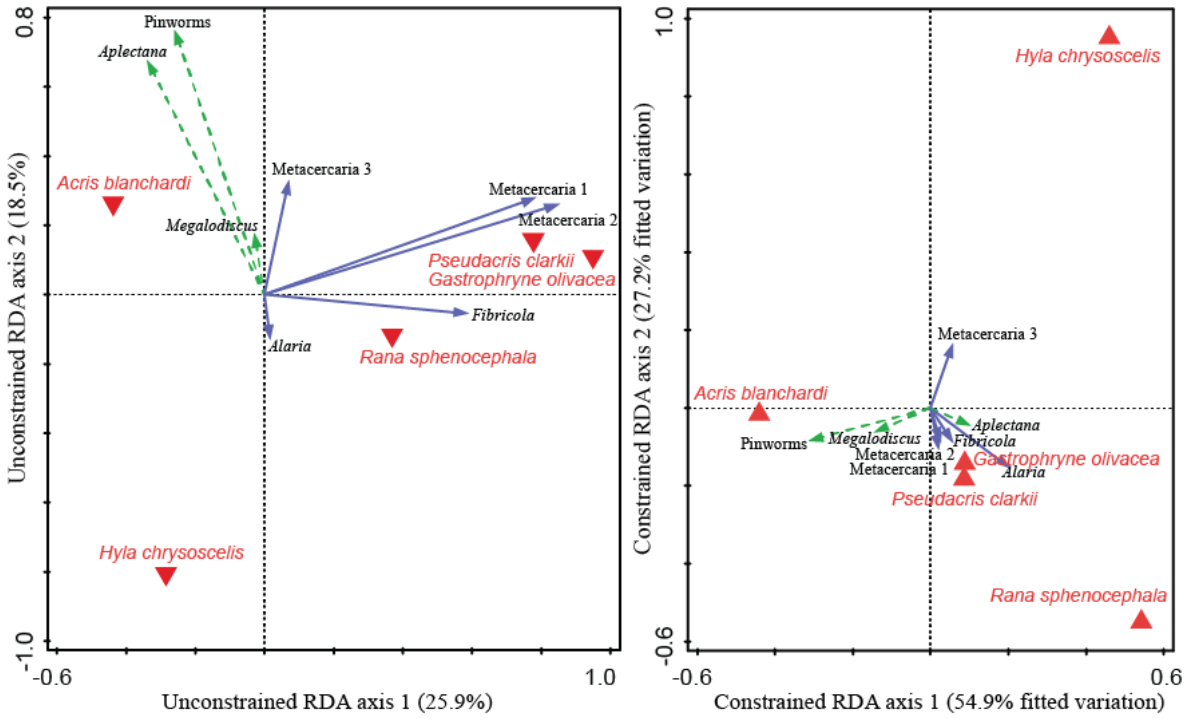

FIGURE 5. Community structure of tadpole parasites, including an (**A**) unconstrained, and a (**B**) constrained ordination. Ingestion-mode parasites are indicated with green dashed arrows whereas skin-penetrating parasites are indicated with blue arrows. Host species (upside-down triangles) are presented in parasite-species space and did not have an influence on the unconstrained RDA (**A**). In the constrained RDA, parasite community structure is constrained to the variation explained by host species after factoring out host size (**B**).

CHAPTER III

DISTRIBUTION AND REPRODUCTIVE STRATEGIES OF *GYRINICOLA BATRACHIENSIS* (OXYUROIDEA: PHARYNGODONIDAE) AND THEIR FUNGAL SYMBIONTS IN TADPOLES OF FIVE ANURAN SPECIES

ABSTRACT: *Gyrinicola batrachiensis* has a direct life cycle; however, female worms have a complex reproductive strategy. Female nematodes in tadpoles of amphibian species with short developmental periods (a few weeks) reproduce parthenogenetically, while those in tadpoles with longer larval developmental periods (months to years), reproduce by haplodiploidy. In this study, my goals were to document host specificity and reproductive strategy of this nematode in tadpoles of five co-occurring amphibian species that varied in their larval developmental periods. Population structure and reproductive strategies of *G. batrachiensis* varied among tadpoles of different amphibian species and was determined by amphibian species, but not necessarily their developmental time. *Gyrinicola batrachiensis* observed in ranid tadpoles with a long developmental period had high mean intensities and confirmed to the haplodiploidy reproductive strategy with males and didelphic females with thick-shelled and thin-shelled eggs present. In contrast, tadpoles of the three hylid anuran species, which varied in their developmental times from long to short, had relatively low mean intensities and

contained both male and female *G. batrachiensis*. However, female worms only produced thick-shelled eggs in these hosts. Importantly, morphological differences existed among female worms recovered from ranids and female worms recovered from hylid tadpoles with long developmental periods. These data strongly suggest that when strains of *G. batrachiensis* are shared by tadpoles of different amphibian species, species-specific differences in the interactions between these nematodes and their amphibian hosts may influence the reproductive strategies of these nematodes. In addition, I document a new trichomycete fungal association on *G. batrachiensis*.

INTRODUCTION

Oxyurid nematodes in the genus *Gyrinicola* Yamaguti, 1938 infect the gastrointestinal tract of tadpole stages of anurans. These pinworms exhibit stadial specificity and metamorphosed anurans are resistant to infection (Adamson, 1981a; Bursey and DeWolf, 1998; Pryor and Greiner, 2004). Currently, five species have been described in the genus and include *G. batrachiensis* (Walton 1929), Adamson 1981 from North America; *G. chabadamsoni* Planade and Bain, 2008 and *G. tba* (Dinnik 1930) from Europe; *G. japonica* Yamaguti 1938 from Japan; and *G. chabaudi*, Araujo and Artigas, 1982, from South America. However, other than these species descriptions, little information is available on the ecology and tadpole host use by these pinworms. Of those, the North American species, *G. batrachiensis*, has been relatively well studied and is considered to have a complex relationship with its tadpole host by increasing the fermentation rate, which ultimately accelerates the tadpole's developmental time to metamorphosis (Pryor and Bjorndal, 2005).

Adamson (1981a, b, c, d) examined the life cycle, morphology, genetics, and seasonal recruitment of *G. batrachiensis* in tadpoles of eight species of anurans from Canada. His work indicates that *G. batrachiensis* is haplodiploid and females possess 2 sets of chromosomes (2n), whereas males possess 1 set of chromosomes (n). Female worms have a complex reproductive anatomy. One uterine horn produces thick-shelled unembryonated eggs used as transmission agents from tadpole to tadpole, whereas the second uterine horn produces thin-shelled eggs with juveniles used for autoinfection. All thin-shelled autoinfective eggs cannot survive outside of the tadpole host and die within an hour in pond water. Importantly, it has been suggested that the development of the genital tract producing thin-shelled autoinfective eggs varies according to the amphibian species and its larval developmental time (Adamson, 1981d). In tadpoles of some amphibian species with short developmental times, such as true toads (Bufonidae), female nematodes reproduce parthenogenetically and males are absent. Females exhibiting this reproductive strategy are monodelphic, possessing a single uterine horn, and only produce thick-shelled, environmentally resistant eggs that are shed via the host's feces. In contrast, in tadpoles of amphibian species with long developmental times, such as true frogs (Ranidae), both male and female nematodes are present and reproduce via haplodiploidy. Females exhibiting this reproductive strategy are didelphic, and produce thick-shelled and thin-shelled autoinfective eggs within different branches of the uterus. These dramatically different reproductive strategies in *G. batrachiensis* are considered to be adaptive responses to the different life history and developmental strategies of different host species (Adamson, 1981d).

In North America, *G. batrachiensis* has been reported from tadpoles of 16 anuran species, representing four families (Bufonidae, Hylidae, Microhylidae, and Ranidae), from California, Florida, Michigan, Nebraska, and Ohio, as well as Ontario and Quebec, Canada (Walton, 1929, 1933; Pryor and Greiner, 2004; Holoman, 1969; Rhoden and Bolek, 2011; Bursey and DeWolf, 1998; Adamson, 1981a, b, c, d). However, to date only a single study examined the occurrence and reproductive strategies of *G. batrachiensis* from tadpoles of co-occurring anuran species that differ in their developmental time (Rhoden and Bolek, 2011).

Rhoden and Bolek (2011) examined co-occurring tadpoles of *Rana pipiens* with long developmental times and tadpoles of *Bufo woodhousii* with short developmental times for the presence and reproductive strategies of *G. batrachiensis*. As expected, female *G. batrachiensis* infecting tadpoles with long developmental periods were didelphic, produced thin- and thick-shelled eggs, and male and female worms were present. In contrast, female worms only produced thick-shelled eggs in tadpoles with short development, but unexpectedly both male and female *G. batrachiensis* were present. Rhoden and Bolek (2011) suggest that when the haplodiploidy strain of *G. batrachiensis* is shared by tadpoles of different amphibian species that differ in developmental period, strains of these nematodes only produce thick-shelled eggs in species of amphibian tadpoles with a short developmental period. However, little information is available on how often this reproductive strategy occurs, and in what species of amphibians, as no other studies exist on the reproductive strategies of *G. batrachiensis* in tadpoles of co-occurring anuran species. Therefore, my goals for this study were to document *G. batrachiensis* in Oklahoma, examine field host specificity,

and reproductive strategy of this nematode in tadpoles of five co-occurring anuran species that varied in their developmental time.

Additionally, this nematode species has been found to harbor fungal symbionts attached to the cuticle in tadpoles collected from Florida and Louisiana (Pryor and Greiner, 2004; B. Font, personal communication). However, this relationship has been poorly studied in the past, as identification of the fungus and its effects on the pinworms are currently unknown. Thus, I report a relatively new fungal-pinworm association and the distribution of this symbiont on *G. batrachiensis* from different tadpole host species.

MATERIALS AND METHODS

Anuran surveys and worm identification

During May – July 2015 and April – June 2016, a total of 162 tadpoles of five anuran species were collected from a small pond at the Teal Ridge wetland located in Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405). These included southern leopard frogs, *Rana sphenocephala* (Family: Ranidae), Cope's gray treefrogs, *Hyla chrysocelis*, Blanchard's cricket frogs, *Acris blanchardi*, spotted chorus frogs, *Pseudacris clarkii* (Family: Hylidae), and Great Plains narrow-mouthed toads, *Gastrophryne olivacea* (Family: Microhylidae). Tadpoles were transported to the laboratory in 19 L buckets filled with pond water and euthanized with MS-222 (tricaine methanesulfonate) within 72 hours of capture. All tadpoles were identified using keys by Altig et al. (2008) and Altig and McDiarmid (2015). Snout-vent length (SVL), total length (TL), tail height (TH) and Gosner stage (GS) was recorded for tadpoles according to Gosner (1960) and McDiarmid and Altig (1999).

During necropsy, the complete digestive tract was removed from each tadpole, placed in an individual Petri dish in aged tap water, the digestive tract was gently pulled apart with forceps and the content was examined with an Olympus SZ61 stereomicroscope for the presence of *G. batrachiensis*. All nematodes were removed and fixed in 70% ethanol, cleared in glycerol, and identified according to Adamson (1981a). Pinworms were then individually placed on slides with glycerol, covered with a coverslip and examined for morphological characteristics and fungal symbionts (see below) with an Olympus BX-51 upright research microscope configured for bright field and differential interference contrast microscopy (ICM) with plain fluorite objective for parasites. Prevalence, mean intensity (MI), and mean abundance (MA) of pinworms for each anuran species was calculated according to Bush et al. (1997). Because variances were heteroscedastic, the Kruskal–Wallis test and the Dunn's multiple comparison tests were used to compare differences in MA and MI of *G. batrachiensis* among different anuran species. Additionally, Pearson's correlation coefficient was used to determine relationships among tadpole SVL, TL, TH and intensity and abundance for *G. batrachiensis*. Spearman's rank correlations were used to determine these relationships with tadpole GS rank. The Šidák correction was used to correct for the number of correlations conducted.

Pinworms from each host individual were identified as juveniles or adults. Adult pinworms were sexed and measurements were taken with a calibrated ocular micrometer for total length, maximum width, nerve ring, esophagus length, pharyngeal bulb length and width, excretory pore, and tail length for adult male and female worms according to Rhoden and Bolek (2011). Additionally, for female pinworms, the location of the vulva

from the anterior end and egg length and width of thick-shelled eggs were measured, while spicule length was measured in male pinworms (Fig. 1). Gravid female *G. batrachiensis* were differentiated as monodelphic or didelphic based on descriptions in Adamson (1981a, b) and the presence of fully developed thick-shelled and thin-shelled eggs (Fig. 1) was recorded for each gravid female pinworm. A one-way analysis of variance (ANOVA) and Fisher LSD post hoc tests were used to compare differences of morphological characters of adult male and female worms recovered from tadpoles of different anuran species. The Kruskal–Wallis test and the Dunn's multiple comparison tests were used when variances were heteroscedastic (Sokal and Rohlf, 1981). Statistics were conducted using STATISTICA v10 (StatSoft, Inc. 2011). All measurements are reported as an average ± 1 SD.

Principle Components Analysis

To visualize morphological differences in adult male and adult gravid female pinworms, a Principle Components Analysis (PCA) was implemented in CANOCO 5 using default settings (Lepš and Šmilauer, 2003). Male analysis was conducted on all measurements, excluding excretory pore and nerve ring, because these structures could not be located in all adult male individuals. Female analysis was only conducted on total length, esophageal length, pharyngeal bulb width and length, and tail length, because these morphological characteristics were significantly different among female worms recovered from different anuran species. Finally, because only a single adult male and single adult female *G. batrachiensis* were recovered from *P. clarkii*, this anuran species was removed from the analyses. A total of 117 female and 34 male *G. batrachiensis* from three anuran species were used in the analyses. The assumption of PCA is that the total

variance of a variable reflects the sum of explained error variance (Grimm and Yarnold, 1995).

Distribution of fungal symbionts on *G. batrachiensis*

Finally, to examine nematodes for the presence and distribution of fungal symbionts, all adult male, female, and juvenile *G. batrachiensis* were examined with an Olympus BX-51 upright research microscope as previously described. Digital images were recorded using a 5 megapixel Olympus digital camera and the morphology of thalli and sporangiospores were recorded and compared to fungal keys and descriptions in Misra and Lichtwardt (2000) and Misra and Horn (2001). Additionally, a male and a female worm with visible fungal symbionts were examined by scanning electron microscopy (SEM). For SEM, both worms were dehydrated in a graded series of ethanol, dried using hexamethyldisilazane, mounted on aluminum stubs, coated with gold palladium, and examined with a FEI Quanta 600 field emission gun ESEM with Evex EDS and HKL EBSD, following protocols in Szmygiel et al. (2014). Prevalence of fungal symbionts is reported for pinworms from each amphibian species, and the distribution of the fungal symbiont on the anterior, mid-body and posterior regions was recorded. The anterior region was defined as the anterior end of the body to the excretory pore; the posterior region was defined from the posterior end of the tail to the cloaca; whereas the mid-body region was defined between the anterior and posterior body regions.

RESULTS

Anuran surveys

A total of 162 tadpoles of five anuran species were collected, and included 32 *R. sphenocephala*, 25 *H. chrysoscelis*, 72 *A. blanchardi*, 26 *P. clarkii*, and 7 *G. olivacea*. Tadpoles of *R. sphenocephala* had the smallest GS rank, and longest mean SVL and longest mean TL. Tadpoles of *G. olivacea* had the shortest mean SVL and tadpoles of *P. clarkii* had the shortest mean TL (Table I). Finally, tadpoles of *H. chrysoscelis* had the highest mean TH; whereas tadpoles of *P. clarkii* had the shortest mean TH (Table I). The Kruskal–Wallis test indicated that significant differences in GS, SVL, TL, and TH existed among the five species of anurans (Table I). The Dunn's multiple comparison tests indicated that *R. sphenocephala* was the only species with a significantly smaller GS rank ($P < 0.02$) than all other combinations of tadpoles; whereas all other treatment groups were not significantly different in GS rank ($P > 0.05$; Table I). Additionally, the Dunn's multiple comparison tests indicated that *R. sphenocephala* had a significantly greater SVL than *A. blanchardi*, *P. clarkii* and *G. olivacea*. In addition, *H. chrysoscelis* had a significantly greater SVL than *P. clarkii* and *G. olivacea*; whereas all other treatment groups were not significantly different in average SVL ($P < 0.01$ for all significant differences; Table I). Similar relationships were observed for TL ($P < 0.05$ for all significant differences) with the exception that there was no significant difference in TL for *R. sphenocephala*, and *H. chrysoscelis* (Table I). Finally, the Dunn's multiple comparison tests revealed that both *H. chrysoscelis* and *R. sphenocephala* had a significantly greater TH than *A. blanchardi*, *P. clarkii* and *G. olivacea* ($P < 0.008$ for all significant differences; Table I).

***Gyrinicola batrachiensis* distribution and reproductive strategy in tadpoles**

Infections of *G. batrachiensis* occurred in tadpoles of *R. sphenocephala*, *A. blanchardi*, *H. chrysoscelis*, and *P. clarkii*; whereas tadpoles of *G. olivacea* were not infected. Prevalence was highest for *A. blanchardi* (83%) and lowest for *P. clarkii* (15%) while MI and MA was highest for *R. sphenocephala* (9.15 ± 10.83; 3.7 ± 8.14) and lowest for *H. chrysoscelis* (3.23 ± 3.35) and *P. clarkii* (0.73 ± 3.13), respectively (Table II). The Kruskal–Wallis tests indicated that significant differences existed in MA ($H_{3, 155}$ = 32.413, $P < 0.001$) and MI ($H_{3, 87}$ = 7.21, $P < 0.05$) of *G. batrachiensis* among the four infected anuran species (Table II). The Dunn's multiple comparison tests indicated that *R. sphenocephala* had a significantly higher MI of *G. batrachiensis* than *A. blanchardi*, and *H. chrysoscelis (*$P < 0.05$ for all significant differences; Table II). Additionally, the Dunn's multiple comparison tests indicated that *R. sphenocephala* had a significantly higher MA of *G. batrachiensis* than *H. chrysoscelis (*$P < 0.05$ for all significant differences; Table II). All other treatment groups were not significantly different for MI or MA of *G. batrachiensis* ($P < 0.01$ for all significant differences; Table II). Finally, no significant correlations ($P > 0.05$) existed for SVL, TL, TH or GS and *G. batrachiensis* abundance or intensity for any of the amphibian species or all tadpoles examined.

A total of 339 individual *G. batrachiensis* were recovered (Table III). Of those, 50% (169) were juveniles, 38% (129) were adult females and 12% (41) were adult males. Although adult males only comprised 12% of the pinworm population, adult male worms infected some individuals of *R. sphenocephala*, *A. blanchardi*, *H. chrysoscelis*, and *P. clarkii* indicating a haplodiploid reproductive strategy (Table III). All gravid female worms recovered were didelphic and contained thick-shelled eggs. However, thin-shelled eggs were only observed in some female *G. batrachiensis* infecting tadpoles of *R.*

sphenocephala; whereas all gravid females recovered from tadpoles of the other anuran species only contained thick-shelled eggs (Table III).

Morphological comparisons of *Gyrinicola batrachiensis* from different amphibian hosts

Female *G. batrachiensis* recovered from tadpoles of three anuran species were significantly different for five of the eleven morphological characters examined (Table IV). The Dunn's multiple comparison tests revealed that female pinworms recovered from *R. sphenocephala* had significantly larger total length, esophageal length, pharyngeal bulb length and width, and tail length than female pinworms recovered from *A. blanchardi* ($P < 0.05$ for all comparisons; Table IV). In contrast, female pinworms recovered from *H. chrysoscelis* only differed in tail length and had a significantly smaller tail length than female pinworms recovered from *R. sphenocephala* ($P < 0.05$ for all comparisons; Table IV). Finally, no significant differences were observed for any of the nine morphological characters examined for male pinworms recovered from tadpoles of the three anuran species (Table V).

Principle Components Analysis

PCA analysis for female worms shows that principal component 1 (PC1) accounted for 52 percent of the variation, and that the first two components account for 72.73 percent of the variation (Table VI). PC1 had all negative values for all variables and was most negatively weighted by pharyngeal bulb length. PC2 also had all negative values and was most negatively weighted by pharyngeal bulb width. A plot of the canonical function shows that there was graphic separation between female pinworms

recovered from *R. sphenocephala* and *A. blanchardi* (Fig. 2). A slight overlap was seen between *R. sphenocephala* and *H. chrysoscelis*.

PCA analysis for male worms shows that principal component 1 (PC1) accounted for 52.85 percent of the variation, and that the first two components account for 70.91 percent of the variation (Table VII). PC1 had all negative values for all variables and was most negatively weighted by pharyngeal bulb length. PC2 had both positive and negative values and was also most negatively weighted by pharyngeal bulb length. A plot of the canonical function shows that there was graphic separation between male pinworms recovered from *R. sphenocphala*, *A. blanchardi* and *H. chrysoscelis* (Fig. 2).

Fungal symbionts

A total of 225 of 339 (66%) *G. batrachiensis* were infested with fungal symbionts. Sixty-eight percent of adult female, 56% of adult male, and 68% of juvenile *G. batrachiensis* were infested. In terms of amphibian host species, pinworms recovered from *R. sphenocephala* and *A. blanchardi* had a higher prevalence of 72% and 69% of fungal symbionts, respectively, than pinworms recovered from *H. chrysoscelis* and *P. clarkii* which was 44% for pinworms recovered from both amphibian species.

The fungal symbionts grew in clusters of individual thalli attached with a fused holdfast system to the cuticle of *G. batrachiensis*. Clusters of thalli were most commonly observed on the posterior region of nematodes (50%), followed by the anterior region (36%) and least often on the mid-body region (14%). Thalli were dimorphic, contained elongate-ellipsoidal trichospores, and most closely resembled fungi in the class Trichomycetes (Fig. 3).

DISCUSSION

My study is only the second to compare the infection levels and reproductive strategy of *G. batrachiensis* in tadpoles of multiple co-occurring amphibian species that differ in their developmental period. Previous studies by Adamson (1981a), Pryor and Greiner (2004) and Rhoden and Bolek (2011) indicate that this pinworm is a tadpole generalist and has been reported to infect tadpoles of 16 species of anurans in North America. My results expand the geographic range for *G. batrachiensis* in the Great Plains region of North America and add two new host records from tadpoles of *A. blanchardi* and *P. clarkii.* More importantly, my data support the only other study on co-occurring amphibians which indicated that populations of *G. batrachiensis* do not always fit the two alternative reproductive strategies in long and short developmental period tadpoles when tadpoles of those amphibian species co-occur (Rhoden and Bolek, 2011).

Although tadpoles of the five anuran species sampled in this study commonly co-occur in the same body of water, they differ in their developmental time through metamorphosis. Of the five anuran species sampled in this study, tadpoles of *R. sphenocephala* and *A. blanchardi* have a relatively long developmental period (Dodd, 2013; Altig and McDiarmid, 2015). Tadpoles of *A. blanchardi* are predominantly found in ponds throughout the summer months and their developmental time to metamorphosis can range from just over four weeks to as long as12.9 weeks. In contrast, tadpoles of *R. sphenocephala* take 8.5 to 12.5 weeks to develop through metamorphosis and tadpoles of this anuran species can be found in ponds throughout most of the year in the southern part of their range (Dodd, 2013; Altig and McDiarmid, 2015). Tadpoles of *H. chrysoscelis* occur in ponds throughout May and June and have an intermediate time to

metamorphosis of just over three weeks to as long as nine weeks (Dodd, 2013; Altig and McDiarmid, 2015). Finally, tadpoles of the hylid *P. clarkii* and the microhylid *G. olivacea* have the shortest developmental times ranging from as short as under three weeks to as long as 10 weeks (Dodd, 2013; Altig and McDiarmid, 2015). As a result of this relatively short developmental period, tadpoles of *P. clarkii* and *G. olivacea* only co-occur in ponds with other anuran species for a relatively short period of time during the early spring or throughout the summer, respectively (Dodd, 2013; Altig and McDiarmid, 2015).

Previous surveys indicate that tadpoles of various *Rana* spp., *Pseudacris* spp. and *Hyla* spp., are common hosts for *G. batrachiensis* but amphibian species-specific differences in tadpole habitat, behavior, and/or reproductive strategies of these pinworms affect the distribution and intensities of *G. batrachiensis* in tadpoles of different amphibian species (Adamson, 1981a; Pryor and Greiner, 2004; Rhoden and Bolek, 2011). Although in my study, tadpoles of *G. olivacea* were not infected with *G. batrachiensis*, tadpoles of this anuran species are strictly filter feeders and their mouthparts are incapable of rasping algae or other surface detritus (Dodd, 2013; Altig and McDiarmid, 2015). This strongly suggests that the inability of tadpoles of *G. olivacea* to ingest eggs of *G. batrachiensis* prevents them from acquiring infections of *G. batrachiensis*. More importantly, significant differences occurred in MA and MI of *G. batrachiensis* and reproductive strategies of this pinworm among tadpoles of the four other anuran species sampled in this study.

The occurrence of didelphic females and the presence of at least some male individuals of *G. batrachiensis* in tadpoles of the four infected anuran species collected

from Teal Ridge suggest that these anuran species shared the haplodiploid autoinfective strain of *G. batrachiensis*. However, prevalence appears to be controlled by tadpole ecology and life history, while mean intensity appears to be controlled by tadpole physiology, but not necessarily the developmental period of each anuran species. For example, tadpoles of *R. sphenocephala, A. blanchardi,* and *H. chrysoscelis* had relatively high prevalence of this nematode compared to tadpoles of *P. clarkii*. Tadpoles of *R. sphenocephala, A. blanchardi,* and *H. chrysoscelis* are either benthic in their habitats and/or feeding ecology suggesting that tadpoles of these anuran species come into contact with the thick-shelled eggs of *G. batrachiensis* on the pond bottom more commonly (Dodd, 2013; Altig and McDiarmid, 2015). Unfortunately, nothing is known about the ecology of tadpoles of *P. clarkii* (Dodd, 2013; Altig and McDiarmid, 2015). However, studies on tadpoles of other *Pseudacris* species indicate that they swim at or near the surface of the water where they consume algae and may feed less often on the pond bottom, reducing their chance of coming in contact with thick-shelled eggs of *G. batrachiensis* (Dodd, 2013; Altig and McDiarmid, 2015). In contrast, the observed differences in the mean intensities of *G. batrachiensis* among tadpoles of *R. sphenocephala, A. blanchardi,* and *H. chrysoscelis* appear to be more complex.

Tadpoles of *R. sphenocephala* had significantly higher mean intensity of *G. batrachiensis* than tadpoles of *A. blanchardi* and *H. chrysoscelis.* Importantly, some female pinworms recovered from tadpoles of *R. sphenocephala* produced both thin-shelled autoinfective eggs as well as thick-shelled eggs; whereas all gravid female pinworms recovered in tadpoles of *A. blanchardi* and *H. chrysoscelis* only produced thick-shelled eggs. Not surprisingly, this suggests that pinworms in tadpoles of *R.*

sphenocephala increased their intensities through autoinfective thin-shelled eggs; whereas tadpoles of *A. blanchardi* and *H. chrysoscelis* infected with pinworms that did not produce thin-shelled autoinfective eggs had relatively low intensities. However, what was surprising was the fact that these different reproductive strategies of *G. batrachiensis* were not correlated with differences in amphibian species time to metamorphosis.

Previous surveys of *H. chrysoscelis* indicate that tadpoles of this anuran species are commonly infected with the haplodiploid autoinfective strain of *G. batrachiensis*; whereas studies on tadpoles of other *Pseudacris* species indicate they are commonly infected with the parthenogenetic strain of *G. batrachiensis* (Adamson, 1981a). Unfortunately, in this study only 1 and 14 adult gravid pinworms were recovered from tadpoles of *P. clarkii* and *H. chrysoscelis*, and it is unclear if the low sample size of adult gravid pinworms affected the detection of thin-shelled autoinfective eggs in these populations of *G. batrachiensis*. However, gravid female pinworms were commonly observed in the relatively long developing tadpoles of *A. blanchardi*. These observations suggest that species-specific host parasite interactions may play an important role in the reproductive strategies of these pinworms.

Previous studies on nematodes and other helminth parasites indicate that host species can influence worm morphology (Rhoden and Bolek, 2011; Vhora and Bolek, 2013, 2015; Stigge and Bolek, 2015, 2016; Wiles and Bolek, 2015). Therefore, it was not surprising that significant differences existed in adult female pinworm morphology recovered from tadpoles of *R. sphenocephala* and *A. blanchardi.* More importantly, my PCA analysis clearly placed female pinworms recovered from tadpoles of *R. sphenocephala* and *A. blanchardi* in distinct geographical space, strongly suggesting that

host species had a strong effect on pinworm growth and development. Recent studies on size and development of helminth parasites in different species of hosts clearly indicate that helminth size is strongly related to reproduction effort (Stigge and Bolek, 2015, 2016). Taken together, my study suggests that anuran species-specific effects may play a major role on the reproductive strategies of these generalist pinworms in different amphibian hosts.

Finally, populations of *G. batrachiensis* nematodes collected from Teal Ridge were commonly infested with a symbiotic trichomycete fungus. Trichomycete fungi are symbionts of freshwater and marine arthropods and reside attached to the cuticle of the rectum of their hosts and currently only a single trichomycete species (*Enterobryus elegans*) is known to infest the cuticle of a pinworm, *Rhigonema infecta*, which resides in the hindgut of an herbivorous millipede, *Narceus annularis* (Wright, 1979). However, previous reports by Pryor and Greiner, (2004) of *G. batrachiensis* populations from Florida tadpoles indicated that they were typically covered in dense, white flocculent material growing from the cuticle. Additionally, fungi like symbionts on the cuticle of tadpole pinworms are known from *G. batrachiensis* populations from Louisiana (B. Font, personal communication) suggesting that this fungus may be a common symbiont across the range of *G. batrachiensis.* However, currently its effects on these pinworms and their tadpole host parasite interactions are not known.

Clearly, the reproductive biology of *G. batrachiensis* appears to be complex and simple designations of tadpole developmental time may not be useful in predicting these nematodes reproductive strategies. What is needed now are cross-transmission studies in different genera and species of amphibians that differ in their developmental periods, to

determine whether tadpole developmental period or other amphibian genus-specific factors, species-specific factors, or both play a role in the reproductive strategy of this nematode. In addition, future experiments that cross male and female *G. batrachiensis* from different pinworm strains would also be informative in gaining a better understanding of the role of genetics for these alternative reproductive strategies. Such cross infection studies should provide baseline data that will allow future testing of hypotheses into what host factors or reproductive strategies of nematodes selected for parthenogenetic strains of *G. batrachiensis*.

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Table I. Developmental time (DT*) in weeks; average snout-vent length (SVL), total length (TL) and tail height (TH) in mm ± 1SD (range); range of Gosner stages (GS) and values for five species of anuran tadpoles collected from Teal Ridge, Stillwater, Oklahoma. Lowercase letters represent significant differences in mean abundance among host combinations ($P \leq 0.03$ for all significant differences).

	R. sphenocephala (n=32)	*A. blanchardi* (n=72)	*H. chrysoscelis* (n=25)	*P. clarkii* (n=26)	*G. olivacea* (n=7)	Statistic	*P*<
DT	8.5-12.5	4.1-12.9	3.4-9.3	4-6	2.9-10		
SVL	17.92 ± 4.92^{a} (12-29)	13.74 ± 2.36^{b} (7-19)	15.08 ± 1.90^{ab} (11-18)	10.62 ± 2.30^{c} (7-18)	10.07 ± 0.45^{c} (9.5-11)	$H = 66.16$	**0.0001**
TL	40.47 ± 14.05^{a} (21.5-71.5)	32.45 ± 6.13^{a} (16.5-44)	35.00 ± 5.39^{a} (25-46.5)	24.69 ± 7.77^{b} (13-46)	25.21 ± 2.14^{b} (21-28)	$H = 40.72$	**0.0001**
TH	8.92 ± 3.36^{a} (18.5-4.5)	5.99 ± 1.57^{a} (3-9.5)	9.18 ± 1.63^{b} (5.5-12)	5.29 ± 1.83^{b} (2-10)	5.36 ± 0.38^{b} (5-6)	$H = 64.04$	**0.0001**
GS	$26 - 44^{a}$	$26 - 46^{b}$	$30 - 42^{b}$	$29 - 45^{b}$	$36 - 39^{b}$	$H = 32.59$	**0.0001**

*Data referenced in Altig and McDiarmid (2015).

Table II. Prevalence (%), mean intensity (MI), and mean abundance (MA) of *Gyrinicola batrachiensis* from tadpoles of five anuran species collected from Teal Ridge, Stillwater, Oklahoma. Lowercase letters represent significant differences in mean abundance among host combinations ($P < 0.05$ for all significant differences).

Species	% (No. infected/No. examined)	MI ± 1SD (range)	MA ± 1SD
Rana sphenocephala	41 (13/32)	9.15 ± 10.83[a] (1-36)	3.7 ± 8.14[a]
Acris blanchardi	83 (60/72)	3.83 ± 4.03[b] (1-21)	3.19 ± 3.94[b]
Hyla chrysoscelis	52 (13/25)	3.23 ± 3.35[b] (1-10)	1.68 ± 2.88[ab]
Pseudacris clarkii	15 (4/26)	4.75 ± 7.5[ab] (1-16)	0.73 ± 3.13[ab]
Gastrophryne olivacea	0 (0/7)	-	0

*Data referenced in Altig and McDiarmid (2015).

Table III. Number of juveniles, males, and females; uterus type, type of eggs in uterus, and suggested reproductive strategy of component populations of *Gyrinicola batrachiensis* recovered from four species of anuran tadpoles from Teal Ridge, Stillwater, Oklahoma.

Species	No. of Juveniles	No. of Males	No. of Females	Uterus Type	Type of Eggs in Uterus	Reproductive Strategy
Rana sphenocephala	63	13	29	Didelphic	Thick-shelled and thin-shelled	Haplodiploidy
Acris blanchardi	77	23	85	Didelphic	Thick-shelled	Haplodiploidy
Hyla chrysoscelis	16	4	14	Didelphic	Thick-shelled	Haplodiploidy
Pseudacris clarkii	13	1	1	Didelphic	Thick-shelled	Haplodiploidy

Table IV. Major dimensions† (in micrometers, unless otherwise stated) of female *Gyrinicola batrachiensis* (Walton, 1929) Adamson, 1981 from four species of tadpole anuran hosts collected from Teal Ridge, Stillwater, Oklahoma.

	Rana sphenocephala	*Acris blanchardi*	*Hyla chrysoscelis*	*Pseudacris clarkii*	Statistic*	*P**
N	29	84	14	1		
Total length (mm)	3.23 ± 0.72[a]	2.56 ± 0.90[b]	2.69 ± 1.06[ab]	3.84	$H = 10.154$	**$P = 0.0062$**
Maximum width	352.05 ± 79.80	324.20 ± 91.40	372.30 ± 126.63	520.71	$F = 2.065$	$P = 0.1313$
Esophageal length	518.82 ± 67.88[a]	450.90 ± 72.37[b]	472.58 ± 75.10[ab]	449.24	$H = 25.650$	**$P < 0.0001$**
Pharyngeal bulb length	117.60 ± 14.30[a]	101.55 ± 17.14[b]	106.48 ± 17.78[ab]	102.1	$H = 17.363$	**$P = 0.0002$**
Pharyngeal bulb width	134.60 ± 17.12[a]	118.28 ± 21.19[b]	123.61 ± 24.04[ab]	142.94	$H = 11.927$	**$P = 0.0026$**
Nerve ring‡	144.97 ± 17.63	140.44 ± 22.27	140.44 ± 15.73	-	$F = 0.322$	$P = 0.7256$
Excretory pore‡	645.78 ± 139.77	594.19 ± 181.32	613.62 ± 225.88	816.8	$F = 0.446$	$P = 0.6425$
Tail length	726.13 ± 205.03[a]	488.32 ± 96.12[b]	483.88 ± 186.55[b]	530.92	$H = 24.285$	**$P < 0.0001$**
Vulva (mm)‡	1.27 ± 0.26	1.27 ± 0.33	1.18 ± 0.49	-	$F = 0.276$	$P = 0.7597$
Thick-shelled egg length	92.07 ± 8.14	91.71 ± 6.53	94.95 ± 4.93	91.89	$F = 1.004$	$P = 0.3707$
Thick-shelled egg width	49.99 ± 10.52	51.96 ± 6.09	51.56 ± 6.99	45.95	$F = 1.004$	$P = 0.3707$

†Average ± 1SD, ‡Distance from anterior end, **Pseudacris* removed from analyses due to small sample size

Table V. Major dimensions† (in micrometers, unless otherwise stated) of male *Gyrinicola batrachiensis* (Walton, 1929) Adamson, 1981 from four species of tadpole anuran hosts collected from Teal Ridge, Stillwater, Oklahoma.

	Rana sphenocephala	*Acris blanchardi*	*Hyla chrysoscelis*	*Pseudacris clarkii*	Statistic*	*P**
N	13	23	4	1		
Total length (mm)	1.08 ± 0.30	0.97 ± 0.28	0.81 ± 0.26	0.50	$F = 1.491$	$P = 0.2383$
Maximum width	118.20 ± 52.89	108.31 ± 39.24	81.68 ± 33.08	40.84	$F = 1.072$	$P = 0.3528$
Esophageal length	214.41 ± 48.11	195.85 ± 36.29	172.29 ± 35.46	-	$F = 1.868$	$P = 0.1691$
Pharyngeal bulb length	54.98 ± 11.06	51.75 ± 9.49	43.39 ± 9.77	-	$F = 2.039$	$P = 0.1449$
Pharyngeal bulb width	56.16 ± 12.68	51.28 ± 10.97	44.67 ± 8.72	-	$F = 1.725$	$P = 0.1925$
Nerve ring‡	134.84 ± 16.99	120.15	-	-	$F = 0.498$	$P = 0.6088$
Excretory pore‡	349.69 ± 94.82	360.75 ± 5.89	382.875	-	$F = 0.085$	$P = 0.9200$
Tail length	222.92 ± 85.27	202.86 ± 5.89	132.73 ± 51.39	122.52	$F = 1.007$	$P = 0.3766$
Spicule length	46.01 ± 5.01	42.95 ± 7.68	43.39 ± 8.55	37.38	$F = 0.805$	$P = 0.4548$

†Average ± 1SD, ‡Distance from anterior end, **Pseudacris* removed from analyses due to small sample size.

Table VI. Partial tabulation of principal component analysis of correlation matrix of 5 morphological characteristics of female pinworms (only the first 2 principal components (PC) are shown).

Characteristic	PC1	PC2
Total length	-0.8989	-0.2756
Esophageal length	-0.8681	-0.2029
Pharyngeal bulb length	-0.9084	-0.2484
Pharyngeal bulb width	-0.8876	-0.2904
Tail length	-0.7052	0.2224
% Variance	51.78	20.99
Cumulative	51.78	72.76

Table VII. Partial tabulation of principal component analysis of correlation matrix of 7 morphological characteristics of male pinworms (only the first 2 principal components (PC) are shown).

Characteristic	PC1	PC2
Total length	-0.8977	0.0527
Maximum width	-0.9034	-0.0464
Esophageal length	-0.9185	0.0312
Pharyngeal bulb length	-0.9488	-0.0840
Pharyngeal bulb width	-0.9429	0.0047
Tail length	-0.6817	0.0333
Spicule	-0.6386	0.0195
% Variance	52.85	18.06
Cumulative	52.85	70.92

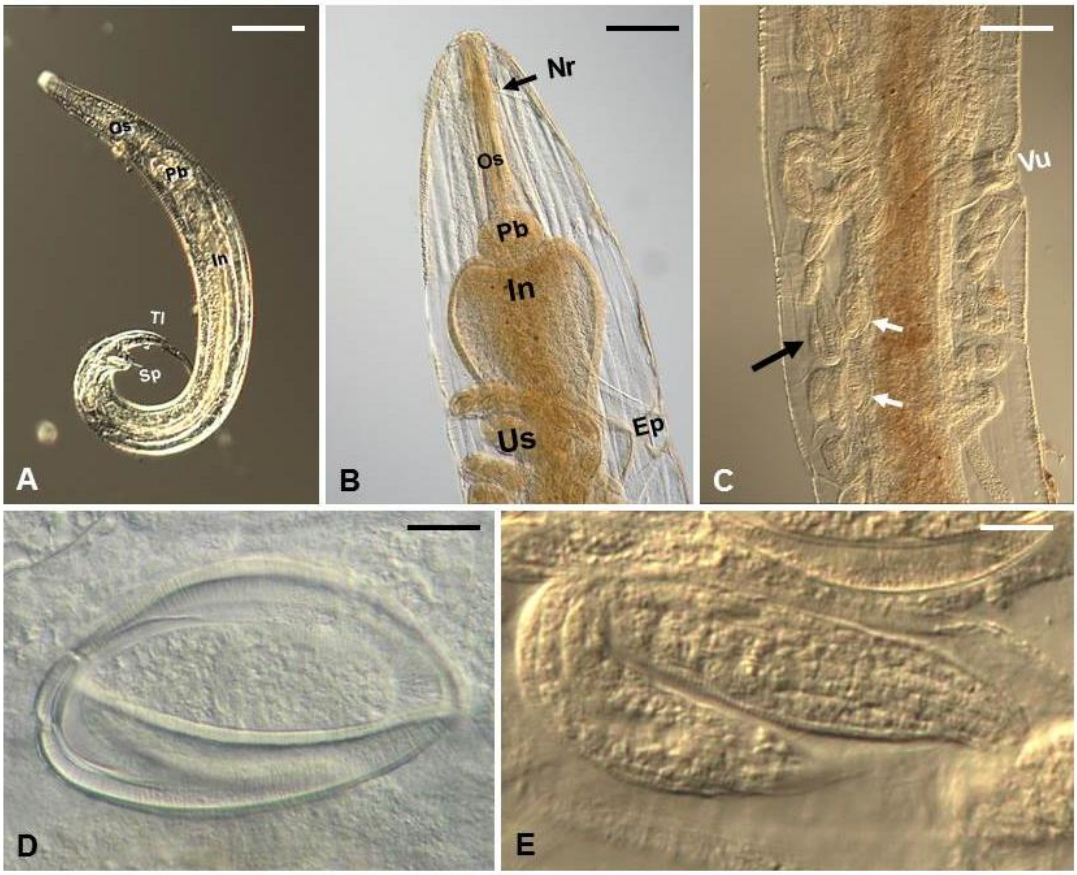

FIGURE 1. ICM photomicrographs of major morphological characters of *Gyrinicola batrachiensis* from a tadpole of *Rana sphenocephala* collected from Teal Ridge, Payne County, Oklahoma. (**A**) Lateral view of entire male worm. Scale bar = 150 µm. (**B**) Anterior end of female worm. Scale bar = 150 µm. (**C**) Dorsal view of mid-body region of a female, showing the vulva, and thick-shelled eggs (white arrows) and thin-shelled egg (black arrow) in the uteri. Scale bar = 40 µm. (**D**) Fully-developed thick-shelled egg in uterus. Scale bar = 20 µm. (**E**) Fully-developed thin-shelled containing a juvenile. Scale bar = 25 µm. Ep = excretory pore, In = intestine, Nr = nerve ring, Os = esophagus, Pb = pharyngeal bulb, Sp = spicule, Tl = tail, Vu = vulva, Us = uterus.

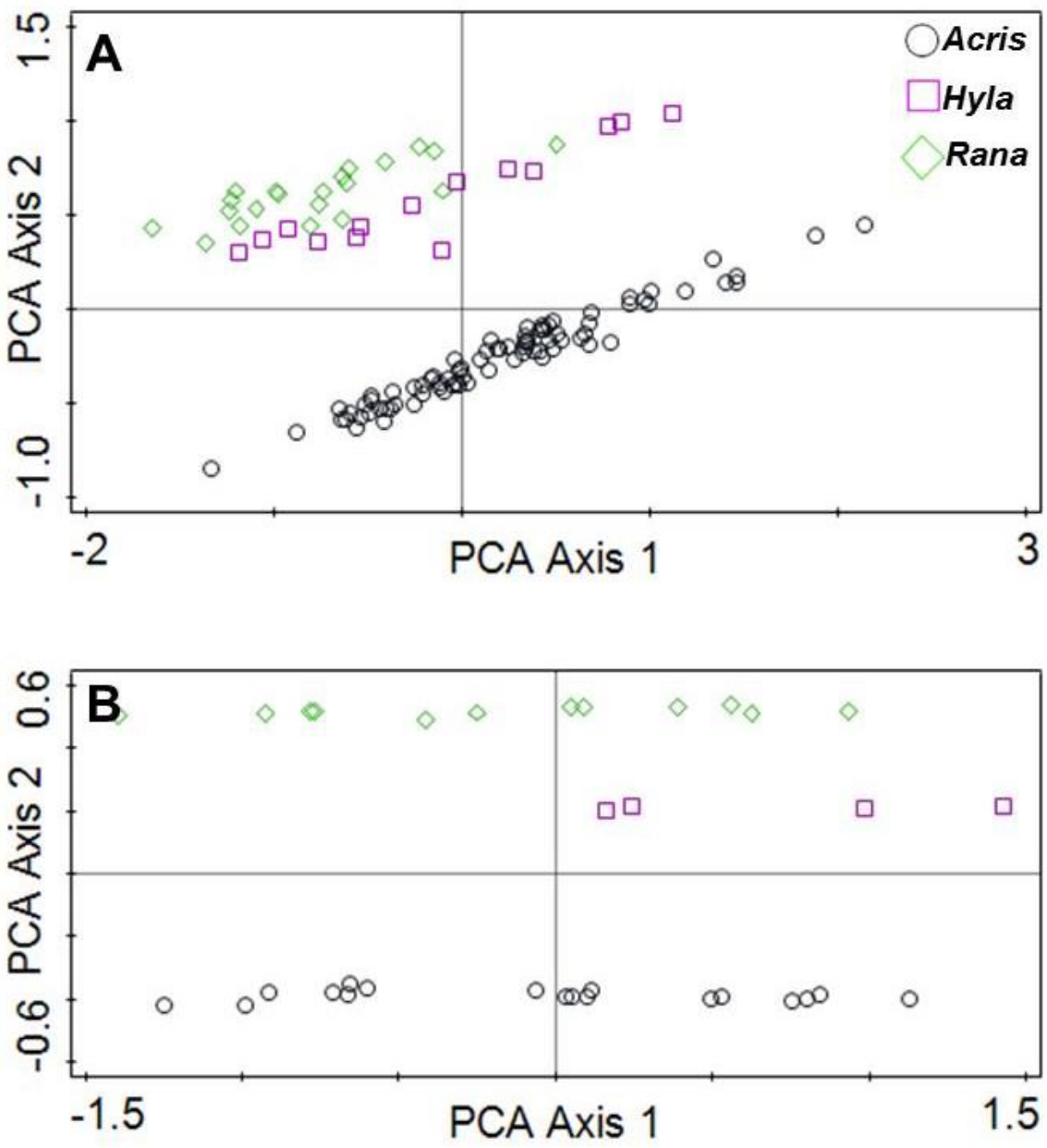

FIGURE 2. Canonical plot of adult female *Gyrinicola batrachiensis* (**A**) and adult male *Gyrinicola batrachiensis* (**B**) recovered from tadpoles of *Rana sphenocephala*, *Acris blanchardi*, and *Hyla chrysoscelis*.

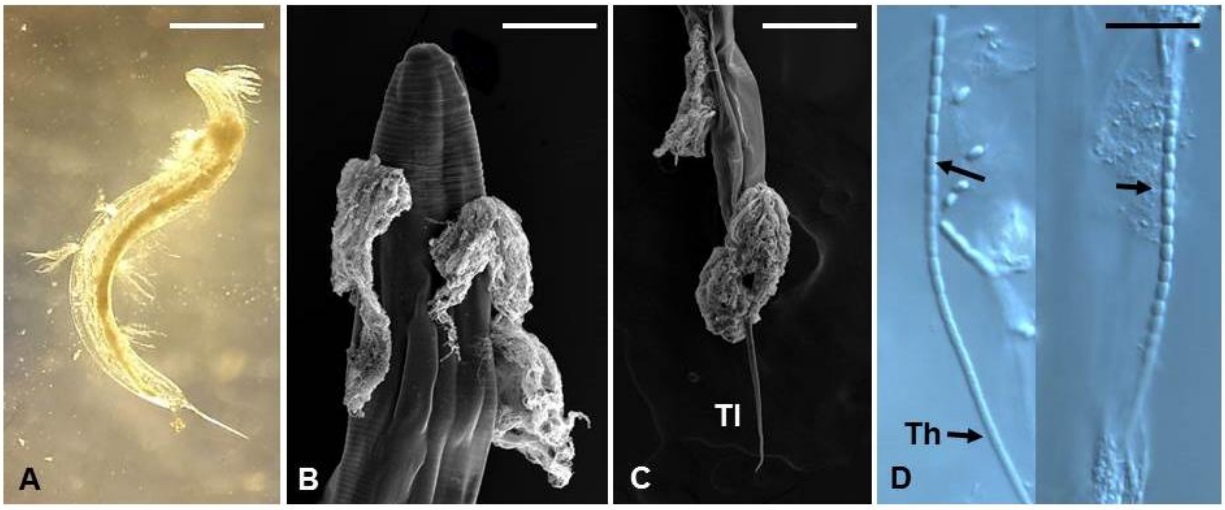

FIGURE 3. Adult female *Gyrinicola batrachiensis* showing attached trichomycete fungal symbiont. (**A**) A live adult female *G. batrachiensis* showing dense clusters of thalli attached on the anterior and mid-body regions of the worm. Scale bar = 500 µm. (**B**) Scanning electron micrograph showing the fused holdfast system attached to the anterior region of *G. batrachiensis.* Scale bar = 150 µm. (**C**) Scanning electron micrograph showing dense clusters of thalli attached to the posterior region of the worm. Scale bar = 150 µm. (**D**) Dimorphic thalli (Th) containing elongate-ellipsoidal trichospores (black arrows). Scale bar = 10 µm.

CHAPTER IV

CONCLUSIONS

Nearly every living organism serves as host to at least one parasite species, suggesting that parasitism may be one of the most common ecological relationships in nature (Bush et al., 2001). Recently, amphibian parasites have been given considerable attention because amphibian declines and extinctions have been documented around the world and some helminth parasites have been linked to some of these declines (Johnson et al., 2002). Previous studies on amphibian parasite life cycles, recruitment, and community structure indicate that host diet, size, and life history play important roles in structuring amphibian helminth communities (Bolek and Coggins, 1998, 2000, 2001, 2003; Hardin and Janovy, 1988; Muzzall, 1991; Muzzall and Peebles, 1991; Gilliland and Muzzall, 1999; Muzzall et al., 2001; Snyder and Janovy, 1994, 1996; McAlpine, 1997; Bolek and Janovy, 2007a, b, 2008; Bolek et al., 2009, 2010; Vhora and Bolek, 2013, 2015; Stigge and Bolek, 2015). However, these studies have focused on adult amphibian parasites and their community structure, with little attention given towards larval stages of amphibians and their parasites (Rhoden and Bolek, 2012, 2015). Thus, the objectives of this thesis were to examine the parasite community structure of tadpoles of sympatric and syntopic anuran species from north Central Oklahoma. This work represents only the

second comparative study on helminth communities in larvae of sympatrically occurring amphibian species. Therefore, data presented within provide a baseline for future studies on helminth community composition of sympatrically occurring anuran tadpoles.

CHAPTER II: THE ROLE OF PARASITE TRANSMISSION STRATEGIES IN STRUCTURING TADPOLE PARASITE COMMUNITIES

In chapter II, I examined the parasite community structure of tadpoles of five sympatrically co-occurring anuran species. This study indicates that helminth communities in anuran tadpoles are not as clearly defined as they are in metamorphosed and adult anurans, which have distinct ecological habitats. While parasite host-specificity cannot be ruled out, my work indicates that anuran tadpole species-specific differences in habitat partitioning and feeding strategies, as well as parasite transmission strategies, play important roles in structuring parasite communities within anuran tadpoles. My data indicate that tadpole size is a primary factor in determining parasite abundances and intensities. However, after controlling for host size, parasite life histories and host species were major factors in influencing parasite community structure within tadpole stages of anurans. A major contribution of this work is that it is only the second comparative study to examine parasite community structure in tadpoles of sympatrically occurring anuran species.

CHAPTER III: HOST SPECIES CAN INFLUENCE GROWTH AND DEVELOPMENT OF HELMINTH PARASITES

In chapter III, my primary objective was to evaluate the distribution and reproductive strategies of the tadpole pinworm, *Gyrinicola batrachiensis*, among tadpoles of different anuran species with varying developmental times. Previous studies indicate that *G. batrachiensis* is commonly found in tadpoles of *Rana* spp., *Hyla* spp., and *Pseudacris* spp.; however, species-specific differences in host habitat, behavior, and/or reproductive strategies of these pinworms affect the distribution and intensities among different host species (Adamson, 1981; Pryor and Greiner, 2004; Rhoden and Bolek, 2011). While four of the five anuran species sampled were infected with *G. batrachiensis* and shared the haplodiploid autoinfective strain of this pinworm, population structure of this pinworm defined as prevalence, mean intensity and reproductive strategy was controlled by the tadpole host's ecology and physiology, respectively. My study suggests that host species-specific differences play a major role on the reproductive strategies exhibited by these pinworms in different amphibian species. Importantly, my data support a previous study on co-occurring amphibians, which indicated that populations of *G. batrachiensis* do not always fit the two reproductive strategies observed in long and short developmental period tadpoles when co-occurring within an environment.

This work provides evidence that helminth communities of larval anurans differ in their structure from adult anurans. However, factors such as tadpole host size, diet, and habitat appear to play an important role in structuring parasite communities within anuran tadpoles, as seen in metamorphosed and adult anurans. Future work is necessary in order to gain a better understanding of other important factors in structuring helminth communities of anuran tadpoles, as well as the influence of host factors on growth and development of some helminth species. I hope my work stimulates interest in this area.

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VITA

Chelcie Cierra Pierce

Candidate for the Degree of

Master of Science

Thesis: PARASITE COMMUNITY STRUCTURE AND REPRODUCTIVE STRATEGIES OF *GYRINICOLA BATRACHIENSIS* IN TADPOLE STAGES OF ANURANS FROM NORTH CENTRAL OKLAHOMA

Major Field: Zoology

Biographical:

Education:
Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in December, 2016.

Completed the requirements for the Bachelor of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in 2013.

Experience:
6 podium and 2 poster presentations at scientific meetings
5 semesters of teaching laboratories for 3 courses, including: Introductory Biology, Animal Biology, Physiology

Professional Memberships:
American Society of Parasitologists
Rocky Mountain Conference of Parasitologists
Southwest Association of Parasitologists