

## HELMINTH AND LEECH COMMUNITY STRUCTURE IN TADPOLES AND CAUDATAN LARVAE OF TWO AMPHIBIAN SPECIES FROM WESTERN NEBRASKA

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**ABSTRACT:** Currently no comparative studies exist on helminth and leech community structure among sympatric anuran tadpoles and salamander larvae. During June–August 2007–2009, we examined 50 bullfrog tadpoles, *Rana catesbeiana*, 50 barred tiger salamander larvae, *Ambystoma mavortium*, and 3 species of snails from Nevens Pond, Keith County, Nebraska for helminth and leech infections. The helminth and leech compound community of this larval amphibian assemblage consisted of at least 7 species, 4 in bullfrog tadpoles and 4 in barred tiger salamander larvae. Bullfrog tadpoles were infected with 2 species of nematodes (*Gyrinicola batrachiensis* and *Spiroxys* sp.) and 2 types of metacercariae (*Telorchis* sp. and echinostomatids), whereas barred tiger salamander larva were infected with 1 species of leech (*Placobdella picta*), 2 species of adult trematodes (*Telorchis corti* and *Halipegus* sp.), and 1 species of an unidentified metacercaria. The component community of bullfrog tadpoles was dominated by helminths acquired through active penetration, or incidentally ingested through respiratory currents, or both, whereas the component community of larval salamanders was dominated by helminths acquired through ingestion of intermediate hosts ( $\chi^2 = 3,455.00$ ,  $P < 0.00001$ ). Differences in amphibian larval developmental time (2–3 yr for bullfrog tadpoles versus 2–5 mo for salamander larvae), the ephemeral nature of intermediate hosts in Nevens Pond, and the ability of bullfrog tadpole to eliminate echinostome infections had significant effects on mean helminth species richness among amphibian species and years ( $t = 12.31$ ,  $P < 0.0001$ ;  $t = 2.09$ ,  $P = 0.04$ ). Differences in herbivorous and carnivorous diet and time to metamorphosis among bullfrog tadpoles and barred tiger salamander larvae were important factors in structuring helminth communities among the larval stages of these 2 sympatric amphibian species, whereas size was important in structuring helminth and leech communities in larval salamanders, but not in bullfrog tadpoles.

Aho (1990) reviewed the literature on amphibian helminth community structure and he concluded that helminth communities of amphibians are highly variable, depauperate, and noninteractive in structure. More recently comparative studies on amphibian helminth life cycles, recruitment, and community structure in anuran hosts by Muzzall (1991b), Muzzall and Peebles (1991), Snyder and Janovy (1996), Yoder and Coggins (1996), McAlpine (1997), Bolek and Coggins (1998a, 2000, 2001), Gilliland and Muzzall (1999), Muzzall et al. (2001), Bolek and Janovy (2007a, 2007b, 2008), Bolek et al. (2009, 2010) have provided baseline data on the community structure, distribution, demography, field host specificity, and life history of anuran helminths. Likewise, Muzzall (1990, 1991a), Muzzall and Schinderle (1992), Bolek (1997), Bolek and Coggins (1998b), Bursey and Schibli (1995), and Muzzall et al. (2003) have provided similar data for caudatan parasites. Though many of these studies have documented amphibian helminth community structure at the infra, component, and compound community level, only Bolek and Coggins (2003) and Yoder and Coggins (2007) have made comparisons between sympatric anuran and caudatan hosts. These studies indicate that salamanders are less commonly infected by helminth species than are anurans. Explanations for lower helminth species richness in caudatans as compared to anuran species include smaller body size, smaller gape size, lower vagility, and more fossorial habitat preference of caudatans than anuran species as well as ecological and physiological host specificity differences (Muzzall, 1991a; Muzzall and Schinderle, 1992; Bolek and Coggins, 2003; Yoder and Coggins, 2007). However, both of these studies focused on helminth community structure of metamorphosed amphibians and few investigators have examined helminth community structure of larval amphibians (Muzzall, 1991a; Muzzall and Schinderle, 1992; Kehr and Hamann, 2003). Because co-occurring

salamander larvae and anuran tadpoles overlap in their body sizes, vagility, and habitat preferences more so than their metamorphosed counterparts, examining these life stages can help us advance our understanding of amphibian helminth community structure.

Tadpoles, the ephemeral, feeding, and nonreproductive larvae of anurans differ significantly in their biology from all other larval and metamorphosed amphibians. Temperate-zone anuran tadpoles are found in streams, ponds, or ephemeral bodies of water, where they feed on suspended and/or epibenthic algae. Tadpole digestive systems are dramatically different from those of predatory adult frogs and go through an ontogenetic niche shift during development. Tadpoles thus are considered vertebrate analogs to larvae of holometabolous insects (McDiarmid and Altig, 1999). Most salamander larvae are also aquatic and reside in streams, ponds, or ephemeral bodies of water. However, in contrast to tadpoles, salamander larvae are predators that feed on aquatic invertebrates and their digestive systems are more similar to adult amphibians with less dramatic changes occurring during metamorphosis (Tilley, 1964; Petranka, 1998). These differences in life histories of anuran and caudatan larvae have stimulated numerous ecological studies on competition among sympatric tadpoles and salamander larvae, yet no comparative studies exist on helminth community structure among co-occurring tadpoles and larval salamanders (Wilbur, 1972, 1976, 1980; Wilbur and Collins, 1973).

In Nebraska, tadpoles of North American bullfrogs, *Rana catesbeiana* Shaw 1802, and larvae of the barred tiger salamanders, *Ambystoma mavortium* (Green, 1825), occur sympatrically (Fogell, 2010). Bullfrogs have the largest tadpoles of any North America anuran species and are slow to develop, staying in the tadpole stage for 2–3 yr. Bullfrog tadpoles feed on algae and aquatic plan material by scraping algae and plants from substrata, or by filtering algae from the water column (Bolek and Janovy, 2004; Lannoo, 2005). In contrast, larvae of barred tiger salamanders metamorphose within 2–5 mo posthatching, and feed on small invertebrates including, but not limited to, ostracods, aquatic insects, and mollusks (Petranka, 1998). In this

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study, we examined the helminth community structure and leech distribution on bullfrog tadpoles and larval barred tiger salamanders from a small cattle tank overspill pond in western Nebraska. In addition, we examined larval trematode infections in 3 species of aquatic gastropods from this pond, which are sources of metacercariae and adult trematode infections to larval amphibians. Because the larvae of these 2 amphibian species differ in body size and shape, time to metamorphosis, and diet, we were interested in elucidating what role, if any, amphibian time to metamorphosis, size, and differences in their herbivorous or carnivorous diet had in determining helminth and leech communities in these larval amphibians. Finally, we compared characteristics of helminth community structure of these larval amphibians to other studies on larval and adult anuran and caudatan helminth communities.

## MATERIALS AND METHODS

### Description of field site and amphibian surveys

Nevens Pond is a small fishless cattle tank overflow pond located in the Sand Hills of Keith County, Nebraska (41°12.426', 101°24.510'). The pond is approximately 44.5 m long by 20 m wide and has a maximum depth of 2 m. During June–August of most years, Nevens Pond is surrounded by sedges (*Carex* spp.), and bulrushes (*Scirpus* spp.). Depending on the year, daily wind conditions, or cattle use, 20–80% of the surface area of the pond is covered by hornworts (*Ceratophyllum demersum*), pondweeds (*Potamogeton* spp.), and duckweed (*Lemna* spp.). The pond has a rich invertebrate community of turbellarians, annelids, gastropods, bivalves, and arthropods (see Bolek and Janovy, 2007a, 2007b; Bolek et al., 2009, 2010). The dominant snail species during the summer months in Nevens Pond consist of *Physa gyrina* and *Gyrualus parvus* and occasionally *Planorbella trivolvis*, whereas during fall and winter *Promenetus exacuous* also occurs in the pond. Two amphibian species are found at Nevens Pond throughout the year. Bullfrogs and their tadpoles are permanent residents of Nevens Pond and colonized Nevens Pond sometime after 1996 (Snyder, 1996; Bolek and Janovy, 2007a). Barred tiger salamanders breed most years in Nevens Pond, and some gilled adults occur in the pond throughout the year.

During June–August 2008–2009 50 bullfrog tadpoles and 50 barred tiger salamander larvae were collected by dip-net from Nevens Pond. As part of this study we attempted to collect both bullfrog tadpoles and barred tiger salamander larvae each year from Nevens Pond. However, during 2008 barred tiger salamander larvae were absent from Nevens Pond. Animals were transported to the laboratory in 18.9-L buckets filled with pond water without snails and killed in MS-222 (tricaine methanesulfonate) within 72 hr of capture. Snout–vent length (SVL), total length (TL), and age (larvae or gilled adults) were recorded for salamander larvae according to Petranka (1998), whereas SVL, TL, and Gosner stage was recorded for tadpoles according to Gosner (1960) and McDiarmid and Altig (1999). Student's *t*-test was used to compare differences in mean SVL and TL between years and/or amphibian species, whereas a Mann–Whitney *U*-test was used to compare differences in Gosner stages among years in tadpoles. Approximate *t* tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). All tissues, including the skin, buccal cavity, gills, digestive tract, body musculature, internal organs, limbs, and tail were examined for helminth parasites and leeches. At necropsy, each organ was placed in individual Petri dishes and examined with a stereomicroscope. All musculature was teased apart with forceps for encysted helminths, and all worms were removed. Nematodes were fixed in 70% ethanol, cleared in glycerol, and identified as temporary mounts, whereas leeches were relaxed in tap water narcotized with 10% ethanol, and preserved in 70% ethanol. Adult trematodes were relaxed in tap water, fixed in alcohol–formaldehyde–acetic acid (AFA) or 70% ethanol, stained with Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam (Pritchard and Kruse, 1982). All metacercariae were removed from amphibian tissues and examined and identified live as wet mounts using a compound microscope. Echinostomatid metacercariae were scored as normal (living) or enclosed in a fibrous capsule

(melanized) of host origin according to Martin and Conn (1990). Some metacercariae were crushed to release worms from their cysts under coverslip pressure, and all metacercariae were fixed in AFA or 95% ethanol and representative specimens were stained and permanent slides prepared.

### Snail surveys

During July–August 2007–2008, 3 species of aquatic snails, including *G. parvus*, *P. gyrina*, and *P. exacuous*, were collected by sampling aquatic vegetation with a dip net. Because large numbers of snail predatory leeches, *Helobdella triserialis*, occurred in Nevens Pond during June–August 2009, no snails were found in the overflow pond. However, because water continually flows into Nevens Pond from the cattle tank and potentially can introduce snails or cercariae, or both, into the pond, we sampled snails in the associated cattle tank during June–August 2009. All snails were brought back to the laboratory and individually isolated in 1.5-ml-well plates filled with aged tap water within 1 hr of collection and observed daily for shedding cercariae for a period of a week. All snails were then crushed and examined for prepatent infections. Cercariae were identified based on the cercariae keys in Schell (1985) and species, genus and/or type descriptions in Krull (1930, 1931), Leigh (1946), Schell (1962), and Bolek et al. (2010). Some intramolluscan stages and cercariae were fixed in AFA and representative stages were stained and permanent slides prepared.

### Community structure, worm identification, and vouchers

Prevalence, mean intensity, and mean abundance were calculated according to Bush et al. (1997). Mean helminth and/or leech species richness is the sum of worm species per individual amphibian, including uninfected individuals, divided by the total sample size (Bolek and Coggins, 2003). The Chi square test for independence was calculated to compare differences in prevalence among years and/or different amphibian species. Student's *t*-test was used to compare differences in mean intensity, mean abundance and mean helminth and leech species richness between years and/or amphibian species. Approximate *t*-tests (*t'*) were calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). Pearson's correlation was used to determine relationships among amphibian SVL and TL and intensity, abundance and species richness of individual helminth and leech species, and overall helminth and/or leech intensity and abundance. Spearman's rank correlation was used to determine these relationships and Gosner stage of tadpoles. All values are reported as the mean  $\pm$  1 SD. All leeches were checked if they contained blood in the gut, and identified with the use of keys by Klemm (1985, 1991) and descriptions provided by Barta and Sawyer (1990). Nematode adults and juveniles were identified based on the original descriptions or redescriptions by Hedrick (1935) and Adamson (1981a). Metacercariae and adult trematodes were identified based on keys in Schell (1985) and to genus or species based on description in Schell (1962), Macdonald and Brooks (1988), and Bolek et al. (2010). Voucher specimens have been deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accession numbers HWML 67090, *Placobdella picta*; 67089, *Spiroxyis* sp.; 66687, *Gyrinicola batrachiensis*; 49227, *Halipegus* sp.; 49732, *Telorchis corti*; 49730, metacercariae of *Telorchis* sp.; 49731, metacercariae of an echinostomatid). A single bullfrog tadpole and single barred tiger salamander larva fixed in 10% neutral buffered formalin was deposited in the Department of Zoology, Collection of Vertebrates, Oklahoma State University, Stillwater, Oklahoma (A 8054, tadpole of *Rana catesbeiana*; A 8055 larva of *Ambystoma mavortium*).

## RESULTS

### Amphibian surveys

Twenty-five bullfrog tadpoles were collected during 2008 (SVL = 42.8 mm  $\pm$  6.26, 25–57 mm; TL = 97 mm  $\pm$  13.81, 60–119; Gosner stage = 26–41), whereas 25 bullfrog tadpoles (SVL = 51.84 mm  $\pm$  3.74, 43–58 mm; TL = 123 mm  $\pm$  10.31, 102–138; Gosner stage = 29–41), and 50 barred tiger salamander larvae (SVL = 47.64 mm  $\pm$  10.48, 30–80; TL = 101.5  $\pm$  18.89, 52–149), but no gilled adult salamanders were collected during 2009. There

TABLE I. Prevalence (%), mean intensity (MI) and mean abundance (MA) of helminths and leeches recovered from tadpoles of bullfrogs, *Rana catesbeiana*, and larvae of barred tiger salamanders, *Ambystoma mavortium*, collected from Nevens Pond during June–August 2008 and 2009.\*

Species	<i>Rana catesbeiana</i> 2008–2009 (N = 50)			<i>Ambystoma mavortium</i> 2009 (N = 50)			Location
	Prevalence %	MI ± 1SD (range)	MA ± 1SD	Prevalence %	MI ± 1SD (range)	MA ± 1SD	
<b>Trematoda</b>							
<i>Telorchis corti</i>	0	—	0	12	5.2 ± 7.1 (1–19)	0.6 ± 2.8	SI
<i>Telorchis</i> sp. metacercaria	98	109.1 ± 74.2 (3–366)	106.9 ± 75	0	—	0	BC, BM BU
<i>Halipegus</i> sp.	0	—	0	4	1 (1)	0.04 ± 0.2	S
Echinostomatid metacercaria	48	7.8 ± 6.2 (2–29)	3.7 ± 5.8	0	—	0	K
Unidentified metacercaria	0	—	0	2	1 (1)	0.02 ± 0.1	BM
<b>Nematoda</b>							
<i>Gyrinicola batrachiensis</i>	54	7.4 ± 6.6 (1–21)	7.4 ± 6.6	0	—	0	LI
<i>Spiroxys</i> sp.	2	3	0.06 ± 0.4	0	—	0	S
<b>Hirudinea</b>							
<i>Placobdella picta</i>	0	—	0	18	1.6 ± 0.7 (1–3)	0.3 ± 0.7	G, SK

\* % = percent (number infected/number examined); BC = body cavity; BM = body musculature; BU = buccal cavity; G = gills; K = kidneys; LI = large intestines; S = stomach; SK = skin; SI = small intestine.

was a significant difference in SVL and TL among tadpoles collected in 2008 and 2009 ( $t = 5.98$ ,  $P < 0.0001$  SVL;  $t = 7.53$ ,  $P < 0.0001$  TL) with tadpoles being longer in SVL and TL during 2009; there was no significant difference among the sum rank of tadpole Gosner stages among years ( $U = 350.50$ ,  $P = 0.46$ ). Additionally, there were significant differences in SVL and TL among the 25 bullfrog tadpoles and 50 barred tiger salamanders collected in 2009, with salamander larvae being significantly longer in SVL and TL ( $t = 3.49$ ,  $P = 0.0008$  SVL;  $t = 6.36$ ,  $P = 0.0001$  TL).

The helminth and leech compound community of this larval amphibian assemblage consisted of at least 7 species, 4 in bullfrog tadpoles and 4 in barred tiger salamander larvae (Table I). Bullfrog tadpoles were infected with 2 species of nematodes (*Gyrinicola batrachiensis* and *Spiroxys* sp.) and 2 types of metacercariae (*Telorchis* sp. and an echinostomatid), whereas barred tiger salamander larvae were infected with 1 species of leech (*Placobdella picta*), 2 species of adult trematodes (*Telorchis corti* and *Halipegus* sp.), and 1 species of an unidentified metacercariae (Table I). Of the 5,785 helminths and leeches recovered, the compound community was dominated by metacercariae 95.7% (5,534), followed by adult and juvenile nematodes 3.5% (204), adult trematodes 0.6% (33), and leeches 0.2% (14).

Of the 50 bullfrog tadpoles examined, 49 (98.0%) were infected with helminths. The component community for tadpoles consisted of 4 helminth species in 2008 and 3 helminth species in 2009 (Table II). The overall mean helminth abundance for all tadpoles collected during 2008 and 2009 was  $104.9 \pm 65.6$  helminths per infracommunity (range = 0–378). Mean helminth species richness was  $2.0 \pm 0.8$  per tadpole. Multiple species infections were common, with 0, 1, 2, 3, and 4 species occurring in 1, 10, 27, 11, and 1 tadpoles, respectively. There were significant differences in mean helminth species richness between years ( $2.2 \pm 0.7$  for 2008 versus  $1.8 \pm 0.8$  for 2009;  $t = 2.09$ ,  $P = 0.04$ ). No significant correlations occurred between overall helminth intensity, overall

helminth abundance, or species richness and SVL, TL, or Gosner stage during 2008, 2009, or both years combined.

During 2008 and 2009, prevalence, mean intensity, and mean abundance were highest for metacercariae of *Telorchis* sp. (Table II). Of the 5,346 *Telorchis* sp. metacercariae recovered, 74% (3,956) were located in the pharyngeal zone and the associated musculature, whereas 26% (1,390) were encysted in the tail. *Gyrinicola batrachiensis* was present both years, with similar prevalence, mean intensity, and mean abundances. In contrast, prevalence and mean abundance of echinostome metacercariae were significantly lower in 2009 than 2008 ( $\chi^2 = 8.01$ ,  $P < 0.05$ ;  $t = 2.40$ ,  $P < 0.05$ ). Fewer tadpoles were infected and fewer echinostome metacercariae were recovered from tadpoles during 2009 than 2008 (Table II). All echinostomes were located in the kidneys, and most metacercariae recovered in 2008 were not encapsulated by the host, whereas a significant proportion of echinostome metacercariae recovered from tadpoles in 2009 were encapsulated and/or dead ( $\chi^2 = 33.87$ ,  $P < 0.001$ ; Fig. 1). Tadpoles infected with echinostomatid metacercariae in 2008 were significantly smaller in SVL ( $42.3 \pm 6.8$  mm), TL ( $95.9 \pm 14.7$ ), and younger (Gosner stage = 27–41) than tadpoles infected with echinostomatid metacercariae during 2009 (SVL =  $52.1 \pm 3.4$ ; TL =  $121.4 \pm 10.4$ ; Gosner state = 35–41;  $t = 3.61$ ,  $P = 0.0015$  for SVL;  $t = 4.165$ ,  $P = 0.0004$  for TL;  $U = 24.5$ ,  $P = 0.025$  for Gosner stage). There were no significant correlations in abundance or intensity for most helminth species recovered and SVL, TL, or Gosner stage during 2008, 2009, or total collection. However, a significant positive correlation existed between tadpole Gosner stage and echinostome metacercariae abundance in 2009 ( $r = 0.47$ ;  $P = 0.02$ ).

Of the 50 barred tiger salamander larvae examined, 16 (32%) were infected with helminths, or leeches, or both. The component community consisted of 2 adult trematodes, 1 unidentified species of metacercaria, and 1 species of leech (Table I). All leeches contained blood in their gut. Overall mean helminth and leech

TABLE II. Prevalence (%), mean intensity (MI), and mean abundance (MA) of helminths recovered from tadpoles of bullfrogs, *Rana catesbeiana* collected during June–August of 2008 and 2009 from Nevens Pond.

Species	Measure of parasitism	2008 (n = 25)	2009 (n = 25)	Statistic	P
<b>Trematoda</b>					
<i>Telorchis</i> sp. metacercariae	Prevalence (%)	100	96	$\chi^2 = 1.02$	$P > 0.05$
	MI $\pm$ 1 SD (range)	94.9 $\pm$ 61.0 (3–263)	123.9 $\pm$ 84.6 (18–366)	$t = -1.38$	$P = 0.17$
	MA $\pm$ 1 SD	94.9 $\pm$ 61.0	118.9 $\pm$ 86.5	$t = -1.13$	$P = 0.26$
Echinostomatid metacercariae	Prevalence (%)	68	28	$\chi^2 = 8.01$	$P < 0.05$
	MI $\pm$ 1 SD (range)	8.3 $\pm$ 7.0 (2–29)	6.6 $\pm$ 3.6 (3–11)	$t = 0.60$	$P = 0.55$
	MA $\pm$ 1 SD	5.6 $\pm$ 7.0	1.5 $\pm$ 3.3	$t' = 2.43$	$P = 0.02$
<b>Nematoda</b>					
<i>Gyrrincola</i> <i>batrachiensis</i>	Prevalence (%)	52	56	$\chi^2 = 0.08$	$P > 0.05$
	MI $\pm$ 1 SD (range)	8.2 $\pm$ 7.21 (1–21)	6.8 $\pm$ 6.1 (1–16)	$t = 0.53$	$P = 0.60$
	MA $\pm$ 1 SD	4.2 $\pm$ 6.6	3.8 $\pm$ 5.7	$t = 0.25$	$P = 0.80$
<i>Spiroxys</i> sp.	Prevalence (%)	4	0	$\chi^2 = 1.02$	$P > 0.05$
	MI $\pm$ 1 SD (range)	3 (3)	–	NA	NA
	MA $\pm$ 1 SD	0.1 $\pm$ 0.6	0	$t = 1.00$	$P = 0.32$

abundances for salamander larvae were  $1.1 \pm 3.09$ . Prevalence was highest for *T. corti* (18%) and lowest, 2%, for unidentified metacercariae (Table I). Mean helminth and leech species richness was low for salamander larvae, being  $0.36 \pm 0.6$  species per individual with 34 salamander larvae infected with 0, 14 salamander larvae infected with 1, and 2 salamander larvae infected with 2 species of helminths and leeches. Significant positive correlations existed for salamander larval SVL and TL and *T. corti* intensity and abundance, *P. picta* abundance, total helminth intensity and abundance, and total helminth and leech intensity and abundance (Table III).

Comparisons among bullfrog tadpoles and barred tiger salamanders collected in 2009 indicated that there were significant differences in species richness and overall helminth and/or leech intensity and abundance between the 2 species of amphibians ( $t = 7.35$ ,  $P < 0.0001$ , species richness;  $t' = 7.25$ ,  $P < 0.0001$ , intensity;  $t' = 7.06$ ,  $P < 0.0001$ , abundance). Individual component communities of these 2 amphibian species were dominated by trematodes. However, bullfrog tadpoles were dominated by metacercariae stages (97%), whereas salamander larvae were dominated by adult trematodes (69%). Significant differences existed in the frequency of all helminths and leeches acquired

through active penetration or attachment (*P. picta*, echinostome, and unidentified metacercaria and metacercariae of *Telorchis* sp. in the tail musculature of tadpoles), incidental ingestion through respiratory currents (metacercariae of *Telorchis* sp. in the pharyngeal zone and associated musculature of tadpoles and *G. batrachiensis*), or ingestion of intermediate hosts (adult *T. corti* and *Haliplus* sp.) among tadpoles and salamander larvae ( $\chi^2 = 3,976.50$ ,  $P < 0.00001$ ).

### Snail surveys

A total of 100 *P. gyrina* and 150 *G. parvus* snails was collected during June–August 2007, 101 *P. gyrina*, 50 *G. parvus*, and 2 *P. exacuus* snails were collected during June–August 2008, and 149 *P. gyrina* snails were collected from the associated well tank during June–August 2009. At least 7 species of larval trematodes infected these snails (Table IV). In 2007, the compound community of *P. gyrina* and *G. parvus* consisted of 6 species of trematodes and no prepatent infections were found, whereas 5 trematode species infected *P. gyrina*, *G. parvus*, and *P. exacuus* in 2008. Additionally, 5 sporocyst and 1 redia prepatent infections were found in *P. gyrina* during 2008. Only a single trematode species infected *P. gyrina* in the well tank during 2009 and no prepatent infections were found. Except for *Haematoloechus* spp., all trematodes were snail specific and no double infections were found in any snail (Table IV). Of the 7 types of cercariae identified, *Haematoloechus* spp., *Haliplus eccentricus*, and *Glythelmins quieta* utilize amphibians as second intermediate and/or definitive hosts, *Telorchis* sp. infects amphibians, snails, and reptiles as second intermediate and/or definitive hosts, whereas echinostomatids and strigeoids use snails, planarians, and amphibians as second intermediate hosts, and birds and mammals as definitive hosts (Schell, 1985; Bolek and Janovy, 2007b; Bolek et al., 2010). Of those, 3 types of cercariae (echinostomatids, *Telorchis* sp., and *H. eccentricus*) concurrently infected tadpoles and/or salamander larvae with metacercariae and/or adult trematode stages. There was no significant difference in the number of *P. gyrina* infected with *Telorchis* sp. and echinostome cercariae during 2007 ( $\chi^2 = 1.84$ ;  $P > 0.05$ ) or 2008, ( $\chi^2 = 2.02$ ;  $P > 0.05$ ), or the number of *P. gyrina* infected with *Telorchis* sp. during 2007 and 2008 ( $\chi^2 = 0.33$ ;  $P > 0.05$ ).

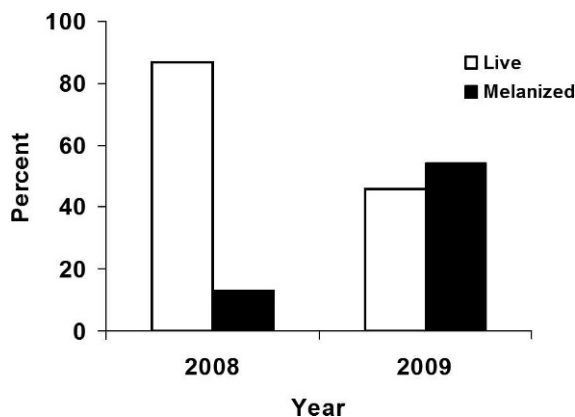


FIGURE 1. Percent frequency of normal and melanized echinostome metacercariae recovered from infected bullfrog tadpoles collected during 2008 and 2009.

TABLE III. Pearson correlation values ( $r$ ) for snout–vent length (SVL) or total length (TL) and intensities or abundances of individual helminth and leech species, total helminths, total helminths and leeches, and helminth and leech species richness for larvae of barred tiger salamanders, *Ambystoma mavortium*, collected in 2009 from Nevens Pond.

	<i>Telorchis corti</i>	<i>Halipegus</i> sp.	Unidentified metacercariae	<i>Placobdella picta</i>	Total helminths	Total helminths and leeches	Helminth and leech species richness
Intensity SVL 2009	0.81*	NA†	NA	0.15	0.79‡	0.50*	–0.25
Intensity TL 2009	0.87*	NA	NA	–0.10	0.82‡	0.60‡	–0.15
Abundance SVL 2009	0.32*	–0.19	–0.01	0.40§	0.31*	0.36‡	0.23
Abundance TL 2009	0.39*	–0.19	–0.07	0.37§	0.37§	0.41§	0.26

\*  $P < 0.05$ .

† NA = not applicable.

‡  $P < 0.02$ .§  $P < 0.01$ .

However, there were statistically significant differences in the prevalence of echinostome and *H. eccentricus* infections in *P. gyrina* among years, with prevalence decreasing significantly in 2008 among both species ( $\chi^2 = 4.12$ ;  $P < 0.05$ , echinostomes;  $\chi^2 = 5.77$ ;  $P < 0.05$ , *H. eccentricus*) and disappearing in 2009.

## DISCUSSION

The major contribution of our paper is the first comparative study of helminth and leech community structure in sympatric larval anurans and caudatans. As in previous studies on helminth parasites in larval amphibians, the helminth and leech community here was depauperate and isolationist in nature. The predatory barred tiger salamander larvae were dominated by indirect life cycle trematodes (*T. corti*) acquired through ingestion of intermediate hosts, whereas herbivorous bullfrog tadpoles were dominated by metacercariae acquired through active cercariae penetration or incidental ingestion of cercariae of *Telorchis* sp. and eggs of *G. batrachiensis*. Previous studies on helminth community structure in larval eastern tiger salamanders, *Ambystoma tigrinum tigrinum*, and larvae and aquatic adult newts, *Notophthalmus viridescens*, from Michigan by Muzzall (1991a) and Muzzall and Schindlerle (1992) support our observations that diet is important in structuring helminth communities. Both of these studies indicate that aquatic life stages of salamanders are commonly infected with adult platyhelminths such as gravid *T. corti* and less often with nongravid adult *Halipegus* sp., acquired via ingestion of intermediate hosts infected with metacercariae

(Schell, 1962; Bolek et al., 2010). In contrast, the only other published study on helminth community structure in anuran tadpoles of *Pseudis platensis* (formally *P. paradoxa*) from Argentina indicates that tadpoles are more commonly infected with metacercariae acquired via active penetration of cercariae. As in our study, the only gravid helminth recovered from tadpoles of *P. platensis* was the nematode *Gyrinicola* sp. through incidental ingestion of eggs, whereas 7 types of metacercariae and 2 nongravid adult trematodes also infected tadpoles of *P. platensis*, suggesting that tadpole helminth communities are dominated by metacercariae (Kehr and Hamann, 2003).

Comparisons of our data with those of Muzzall (1991a) and Muzzall and Schindlerle (1992) also suggest that caudatan size is important in structuring helminth and/or leech communities in aquatic salamanders. As in our study, they found significant positive correlations in salamander length and *T. corti* intensity, and overall helminth intensity, suggesting that host size contributes to an increase in the number or size of intermediate hosts ingested as salamanders increase in length. In contrast to aquatic salamanders, our data indicate that size is not an important factor in structuring helminth communities in bullfrog tadpoles. One reason for this difference may be that bullfrog tadpoles take 2–3 yr to metamorphose and bullfrog tadpoles become loaded with metacercariae. Our data indicate that any helminth intensity and tadpole size relationships may be diluted by intermittent exposure to parasites over a period of 2–3 yr. Additionally, when bullfrog tadpoles are infected with eggs of *G. batrachiensis*, these pinworms autoinfect their host and dilute any size recruitment effects.

TABLE IV. Prevalence (%) of trematode infections in *Physa gyrina*, *Gyraulus parvus*, and *Promenetus exacuous* collected from Nevens Pond during June–August 2007, 2008 and Nevens Well Tank during June–August 2009.

	<i>Physa gyrina</i>			<i>Gyraulus parvus</i>			<i>Promenetus exacuous</i>		
	2007 (n = 100)	2008 (n = 101)	2009 (n = 149)	2007 (n = 150)	2008 (n = 50)	2009 (n = 0)	2007 (n = 0)	2008 (n = 2)	2009 (n = 0)
Echinostomatid cercariae	4%			0%			–		
<i>Glypthelmins quieta</i>		1%	1.7%		0%	–		0%	–
<i>Halipegus eccentricus</i>	8%	1%		0%	0%		–	0%	
<i>Haematoloechus</i> spp.*	0%	0%		52%	56%		–	50%	
Strigeoid cercariae	1%			0%			–		
<i>Telorchis</i> sp.	1%	5%		0%	0%		–	0%	
Sporocysts		5%			0%			0%	
Rediae		1%			0%			0%	

\* Combined prevalence for *Haematoloechus longiplexus* and *Haematoloechus parviplexus* because both species infect *G. parvus* at Nevens Pond (see Bolek and Janovy, 2007b).

Similarly, as in our study, Kehr and Hamann (2003) did not find any significant positive correlations with size and helminth intensity among any of the 11 helminth species they recovered. Importantly, like tadpoles of *R. catesbeiana*, tadpoles of *P. platensis* are large, and have the longest developmental period for any new-world hylid (6 mo), suggesting that host-size and helminth-intensity relationships may be diluted in tadpoles with long developmental periods (Fabrezi et al., 2009). In contrast, as part of a larger study on helminth community structure of tadpoles from Nebraska, Rhoden (2010) examined helminth community structure in tadpoles of 6 species of bufonids, hylids, and ranids from 2 different locations. She found that significant positive correlations existed in helminth intensities and abundance for overall helminths and certain helminth species and tadpole size in amphibian species with a shorter developmental period than tadpoles of *R. catesbeiana* and *P. platensis*. These data support our hypothesis that amphibian species with larval stages with long developmental periods can dilute any relationships with tadpole size and helminth intensities or abundances, particularly when these amphibian larvae are infected with long-lived helminths such as metacercarial stages.

In our study, all helminth and leech species recovered from bullfrog tadpoles and barred tiger salamander larvae were host, or stage, specific. Bullfrog tadpoles were dominated by 2 types of metacercariae (*Telorchis* sp., and echinostomatids) during 2008 and 2009, whereas only 1 barred tiger salamander larva was infected with a single unidentified metacercaria during 2009. This was most likely due to the disappearance of snail first intermediate hosts during 2009, and the survival of most *Telorchis* sp. and some echinostome metacercariae in tadpoles from the previous year. Previous studies on the life history of *T. corti* in mole salamanders, and our unpublished observations from Nevens Pond during 2003, indicate that both types of cercariae encyst as metacercariae in tadpoles, larval salamanders, and snails, whereas echinostomatid cercariae also encyst in planarians (Schell, 1962; Muzzall and Schinderle, 1992; Bolek and Coggins, 2003). In fact, a survey of 5 bullfrog tadpoles and 5 barred tiger salamander larvae from Nevens Pond during 2003 indicated that 100% of bullfrog tadpoles and 100% of barred tiger salamander larvae were infected with *Telorchis* sp. and echinostomatid metacercariae; whereas 100% of barred tiger salamander larvae and 0% of bullfrog tadpoles were infected with *Spiroxys* sp. (Bolek, unpubl. obs.). These data support our hypothesis that differences in amphibian species larval developmental periods and the ephemeral nature of intermediate hosts (snails for trematodes and copepods for *Spiroxys* sp.) in Nevens Pond play a role in helminth community structure in these amphibian species.

Of the 2 types of metacercariae recovered from bullfrog tadpoles, metacercariae of *Telorchis* sp. had the highest prevalence, mean intensity, and mean abundance. In tadpoles, echinostome metacercariae were always site-specific and located exclusively in the kidneys, whereas metacercariae of *Telorchis* sp. were located throughout the musculature, mostly in the pharyngeal region. This was likely due to tadpole respiratory currents incidentally sucking in telorchid cercariae during breathing, or feeding, or both. Recent studies on trematode infections in tadpoles support our observations that some types of cercariae are incidentally ingested by tadpoles through respiratory currents and that some encyst in the pharyngeal region (Bolek and Janovy, 2008; Bolek et al., 2009; Hamann and González, 2009).

At Nevens Pond, both *Telorchis* sp. and echinostomatids used *P. gyrina* snails as the first intermediate host. Therefore, our observations of no significant differences between the prevalence of *Telorchis* sp. and echinostomatid cercariae shed by *P. gyrina* snails during 2007 or 2008 and recovering 5,346 *Telorchis* sp. and 185 echinostomatid metacercariae from bullfrog tadpoles are important. These observations suggest that the higher prevalence and intensities of *Telorchis* sp. metacercariae than echinostomatids may be due to species-specific interactions by cercariae of *Telorchis* sp. and echinostomes with bullfrog tadpoles. Studies indicate that echinostome cercariae actively seek and enter tadpoles via the cloacal opening, then migrate to the kidneys and encyst (Theimann and Wassersug, 2000; Taylor et al., 2004). In contrast, *Telorchis* sp. cercariae will penetrate any body surface (Schell, 1962). Our observations of the high numbers of *Telorchis* sp. metacercariae within the pharyngeal region of tadpoles suggest that bullfrog tadpoles increase their chances of becoming infected with *Telorchis* sp. by mechanically sucking in cercariae, whereas echinostomatid cercariae enter only through the cloaca.

Significant differences were also observed in helminth species richness among tadpoles collected during 2008 and 2009. The lower numbers in 2009 were attributed to lower echinostome infections. Recent studies indicate that prevalence of echinostome metacercariae in tadpoles is stage and/or age dependent, and tadpole mortality only occurs at high-intensity infections in Gosner stage 25 tadpoles (Schotthoefer et al., 2003; Holland et al., 2007; Holland, 2009; Orlofske et al., 2009). Both Schotthoefer et al. (2003) and Holland et al. (2007) indicated that tadpoles of northern leopard frogs, *Rana pipiens*, and green frogs, *Rana clamitans*, were less likely to become infected with echinostome infections as tadpoles aged. Because all our tadpoles were Gosner stage 26 and above, it is unlikely that tadpole mortality can explain the significantly lower echinostome metacercariae infections observed during 2009. In fact, all snail hosts infected with echinostomes disappeared during 2009, suggesting that tadpoles were not exposed to new infections during June–August 2009. This is particularly significant when considering that tadpoles infected with echinostomatid metacercariae in 2009 were significantly larger in SVL and TL and older based on Gosner stage than echinostomatid-infected tadpoles during 2008. In addition, significantly higher numbers of encapsulated and/or dead echinostome metacercariae were found in tadpoles during 2009 than 2008, suggesting that these larger and older tadpoles infected with echinostomatid metacercariae during 2008 were also eliminating established echinostome infections during 2009. Studies by Martin and Conn (1990) on adult northern leopard frogs and green frogs showed that metamorphosed anurans can encapsulate and kill some echinostome metacercariae, whereas Holland (2009) indicated that older green frog tadpoles eliminate echinostome metacercariae within a few hours postinfection; the mechanism underlying elimination is unclear. It is unclear if elimination is due to aging of metacercariae or tadpole encapsulation.

Of the 3 species of adult helminths using amphibians as definitive hosts at Nevens Pond, all complete their life cycles within 2–4 mo, suggesting that cross-infection in bullfrog tadpoles and salamander larvae should occur (Schell, 1962; Adamson, 1981b; Bolek et al., 2010). However, *T. corti* and *Haliplus* sp. infections are specific to salamander larvae, whereas *G. batrachiensis* is specific to bullfrog tadpoles. Recent life-cycle studies of

*Haliplus* spp. by Bolek et al. (2010) indicate that tadpoles of anurans cannot become infected with *Haliplus* spp. because of their inability to feed on odonate paratenic hosts or digest ostracod intermediate hosts; salamander larvae commonly feed on these intermediate and/or paratenic hosts. No comparative host-specificity studies exist for *T. corti* in anuran and salamander larvae. Likewise, no comparative host-specificity studies exist for *G. batrachiensis* in tadpoles or larva salamanders. However, experimental infections of bullfrog tadpoles with *G. batrachiensis* by Pryor and Bjordal (2005) suggest that tadpoles become infected with this pinworm by ingesting *G. batrachiensis* eggs while feeding on the pond bottom. More importantly, Adamson (1981b, 1981c), while conducting field and experimental infection studies, showed that all metamorphosing tadpoles lose their infections of *G. batrachiensis*, and all pinworms die within 24 hr in experimentally infected metamorphosed anurans. These observations suggest that differences in the diet and/or gut physiology of carnivorous amphibians and herbivorous tadpoles may explain the inability of *G. batrachiensis* to establish and survive in carnivorous hosts such as salamander larvae.

Finally, the leech *P. picta* only infested barred tiger salamander larvae; we never observed this species on bullfrog tadpoles. Our observations confirm previous field studies on *P. picta* from Nevens Pond (Bolek and Janovy, 2005) who reported this leech infesting barred tiger salamander larvae and adult bullfrogs, but never bullfrog tadpoles. These observations are surprising, because previous laboratory and field studies report *P. picta* infesting and feeding on bullfrog tadpoles (Barta and Sawyer, 1990; Briggler et al., 2001). In a laboratory study, Barta and Sawyer (1990) exposed bullfrog tadpoles to *P. picta* that had not fed for 1 mo. They observed that as leeches made contact with tadpoles, there was vigorous thrashing and dislodging of some of the leeches in the process. However, most leeches eventually attached to tadpoles and began feeding. They also reported that during feeding, *P. picta* always damaged the skin of tadpoles, creating hematomas, and continued feeding for up to 45 hr. In our study, all leeches recovered from salamander larvae were attached to the gills or front legs, and, although we never found leeches on bullfrog tadpoles, distinct hematomas were seen on the ventral side or tail of 3 tadpoles collected in 2009, suggesting that *P. picta* attach to bullfrog tadpoles on occasion. When barred tiger salamander larvae and bullfrog tadpoles were maintained in the same aquaria, *P. picta* remained attached to salamander larvae for days without causing any apparent distress. These observations suggest that amphibian larval behavior and the presence of external gills as a refuge for leech attachment may be important in structuring *P. picta* populations on these amphibian larvae.

Comparison of our data to helminth-community studies on adult anurans and caudatans indicate that salamander larval helminth communities are similar to other studies on caudatan helminth communities; tadpole helminth communities differ from their metamorphosed anuran counterparts (Muzzall, 1990, 1991a, 1991b; Muzzall and Peebles, 1991; Muzzall and Schindlerle, 1992; Bolek, 1997; McAlpine, 1997; Muzzall et al., 2001; Bolek and Coggins, 2003; Muzzall et al., 2003; Yoder and Coggins, 2007). Comparisons of adult salamanders and salamander larvae indicate that helminth communities in both groups are dominated by parasites with heteroxenous life cycles acquired via trophic dynamics, and with fewer multiple species infections occurring in salamanders. This is not surprising, because other than differences

in habitat, aquatic for larval caudatans and terrestrial and/or fossorial for metamorphosed caudatans, few morphologic or physiologic changes occur during salamander larval development (Tilley, 1964). In contrast, dramatic anatomic, physiologic, and dietary differences exist between metamorphosed anurans and their tadpoles (McDiarmid and Altig, 1999). It is not surprising that these 2 dramatically different anuran life stages also exhibit dramatic differences in their helminth community structure. Importantly, the most similar helminth communities among tadpoles and their metamorphosed anuran counterparts are found among newly metamorphosed anurans that have not had enough time to acquire a typical metamorphosed anuran helminth community (Gilliland and Muzzall, 1999; Bolek and Coggins, 2003). Clearly, tadpoles of anurans and larvae of salamanders are extremely diverse in their biology, habitat, and life histories, as are the adults of these stages; more work needs to be done on the helminth-community structure in larval amphibians.

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