Enteric helminth infections in the red-eared slider turtle *(Trachemys scripta elegans)* from southern Oklahoma

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Abstract: Red eared sliders (n=50) were collected from four southern Oklahoma counties and one northern Texas county and examined for intestinal helminths. Eleven parasite species representing 3 phyla were discovered with no significant differences in parasite load occurring between locations or habitat types. The nematodes *Camallanus* sp, *Spiroxys* sp and the acanthocephalan *Neoechinorhynchus* sp. were the most prevalent helminths collected from the stomach and small intestine. Large intestine was also heavily parasitized primarily by the nematodes *Spironoura* sp*., Camallanus* sp. and the acanthocephalan, *Neoechinorhynchus emydis.* Minimal infections were detected from the urinary bladder and lungs. Total parasite load was positively correlated to carapace length and this relationship was also discovered for some individual species such as *Neoechinorhynchus emydis and N. psuedomydis.* The results of this study were compared to an older parasite study of sliders in Oklahoma with some notable differences in types of parasites, parasite load and organs affected.

Introduction

The red-eared slider (*Trachemys scripta elegans*) is a common, widely distributed turtle in the U.S. (Conant 1975, Ernst et al. 1994), ranging throughout Oklahoma (Sievert and Sievert 1993) and much of central through eastern Texas (Hibbitts and Hibbitts 2016), occupying a variety of aquatic habitats. The species has been studied extensively regarding life history (e.g., Cagle 1950, Gibbons 1990), food habits (e.g., Clark and Gibbons 1969, Hart 1983), nesting biology (e.g., Tucker 2000, Tucker 2001), genetics (e.g., Tucker et al. 1995, Lovich et al 1990, Thomas et al. 2020), and physiology (e.g., Hutton 1957, Reyes and Milsom 2010). However, only a few studies exist that characterize the incidence of parasitic infections in the species. For instance, McAllister and Upton (1988) studied infections of the protozoan *Eimeria* in Texas, Marquardt (1978) studied

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helminth infections in Arkansas, while Harwood (1931), and Everhart (1956, 1957), reported on helminth infections from Oklahoma. Since such a limited amount of data exists on the prevalence of parasitic infections in red-eared sliders from Oklahoma, the focus of this study was to assess helminth infections of red-eared sliders collected from five southern Oklahoma counties and one northern Texas county,

Methods

A total of 50 red- eared sliders were collected from 8 March through 21 August, 2005 from four southern Oklahoma Counties (Pontotoc, n=21; Garvin, n=2, Seminole, n=7, Pittsburg, n=13), and one north Texas county (Jack, n=5). Turtles were collected from one lake (Wintersmith Lake in Pontotoc County, Ok) several farm ponds and roadside canals either by hand or using chicken-wire funnel traps (Iverson 1979) baited with sardines or chicken livers. On capture, each specimen was numbered using the multiple peripheral scute notching method (Cagle 1939), then sexed, measured (plastron length and width) and weighed. After euthanization, each specimen was checked for ectoparasites before the plastron was separated from the carapace by cutting across the bridges. After peritoneal removal and inspection of cavities for cysts or free parasites, the organs were separated and placed in containers with physiological saline. The body cavity was then rinsed with 2% saline and both the washings and shells were examined for parasites. All organs were inspected using methods as described in Everhart (1956) and Schmidt (1971). Histozoic organs (heart, kidneys, spleen, liver, gall bladder) were inspected by teasing apart the tissue under a dissecting microscope and repeatedly washing in saline until clear. Coelozoic organs (alimentary canal, lungs, urinary bladder) were opened by a longitudinal slit to remove any parasites. To loosen any embedded parasites attached to the mucosal wall, a scapel was used for scraping. Lastly, each organ was repeatedly washed in saline and the washing examined for parasites. Total number of individual parasites per host and their specific location was recorded and each was placed in vials of 10% formalin for fixation.

Small to medium-sized trematodes and some hookworms were stained with Lynch's precipitated borax carmine method followed by a fast green counterstain (Galigher and Kozloff 1971). Larger trematodes and most nematodes were stained using Partsch's alum cochineal followed by a fast green counterstain.

Acanthocephalans were stained using Hoyer's Mounting Medium mixed with Giemsa stain (ratio of 19:1) (Everhart 1956, 1957; Galiher and Kozloff 1971), cured in a 45^{o} C oven for 2 weeks, then each slide was sealed with fingernail polish to keep out moisture. Some of the more delicate trematodes were prepared using Hoyer's mounting medium with aceto-carmine stain added to the mixture at a ratio of 1 to 10 parts Hoyer's (Everhart 1956, 1957). All parasite species were identified using the keys of Yamaguti (1958, 1961, 1963a, 1963b), Anderson et al (1974), Daily and Meyer (1996) Amin (2002) and Barger and Nickol (2004).

Before performing statistical analysis on the data using parametric statistical methods, all counts and measurements were log transformed to reduce the effects of unequal variances (Zar 2010).

Results

Eleven species of enteric helminths (from three phyla) were found in 50 turtles (ẍ= 481 per host) (Table 1). An analysis of variance indicated a significant difference in parasite load comparing geographic locations (4 Oklahoma counties, and 1 Texas County) ($P < 0.01$), but no differences in total number of species between locations (P=4.01). There was also no significant difference comparing aquatic habitats (lake vs pond vs roadside canals) in total parasites species per host ($P > 0.248$), and total number of species within each habitat (P= 0.051). A regression analysis showed no correlation of overall parasite abundance to date of capture (P= 0.514), but total number of parasite species infecting hosts was correlated to date of host capture (R^2=0.04, F=0.15, $P < 0.05$). In comparing parasite prevalence between male (n=19) and female (n=31) turtles, there was no significant difference (X^2= 5.08, P= 0.6507), even though some of the most

heavily parasitized individuals were females.

Table 1. Prevalence, mean densities and organ infestations of parasitic helminths from *Trachemys scripta elegans* from Oklahoma (n= 4 counties, 45 turtles) and Texas Counties (n=1 county, 5 turtles).

Parasite	no. collected	% infected turtles	x ± SE	primary organ
Acanthocephala				
Neoechinorhynchus psuedomydis	12,937	57	453.9 ±392.6	small intestine (99.7%)
Neoechinorhynchus emydis	5,154	63	163.6 ±196.4	small intestine (99.0%)
Nematoda				
Camallanus sp.	3,062	92	66.6 ± 78.7	small intestine (94.2%)
Spiroxys sp.	1,278	78	32.0 ±40.3	stomach (92.2%)
Spironoura sp.	1,101	47	45.9 ±66.9	large intestine (67.1%)
Strongyloides sp.	1	2	1.0 ±0.5	small intestine (100%)
Digenea				
Telorchis sp.	456	22	41.5 ±64.7	small intestine (99.5%)
Schizamphisomoides sp.	24	14	3.4 ±1.2	large intestine (83.3%)
Spirorchis emydis	6	10	1.2 ±0.5	lungs (100%)
Diarmostorchis blandingi	3	2	3.0 ± 1.5	lungs (100%)
Monogenea				
Polystomoides coronatum	24	16	3.0 ±3.3	bladder (100%)

Nematodes such as *Camallanus sp.* (92%) and *Spiroxys sp.* (78%) and acanthocephalans of the genus *Neoechinorhynchus* (2 species) (60%) were the most prevalent enteric parasites, followed by the nematode *Spironoura sp.* (47%), a digenetic trematode, *Telorchis sp.* (22%), a monogenetic trematode, *Polystomoides sp*. (16%) and the digenetic trematode, *Schizamphistomoides sp.* (14%). All other parasites identified, *Spirorchis sp., Diarmostorchis sp.,* and *Strongyloides sp.* were recovered from 10% or less of the hosts sampled. All 50 turtles examined were infected with at least one parasite species, and all but two had multi species infections (range 2-8). The small intestine was the most heavily infected organ (n=21,797), primarily with *Neoechinorhynchus psuedomydis* (59.1%), *Neoechinorhynchus emydis* (23.3%) and *Camallanus sp*. (13.2%) (Table 1), and contained the highest number of parasites species per host (ẍ=3.0; range 2-5). The acanthocephalans, *Neoechinorhynchus emydis* and *N. psuedomydis* co-inhabited 28% of hosts with a heavy burden of individuals in the small intestine. However, the presence of one did not appear to affect the presence of the other as there were no difference in the population sizes of the two species in the intestines of co-inhabited turtles (t=0.429, P > 0.05).

The stomach was also heavily parasitized (n=1,355) with 80.4% of turtles containing stomach worms (ẍ=33), primarily *Spiroxys sp*. (87.0%), *Camallanus sp.* (12.3%), and *Neoechinorhynchus emydis* (0.51%). The large intestines contained 860 worms (ẍ=27), primarily *Spironoura sp*. (85.8%), *Neoechinorhynchus emydis* (5.3%), *Neoechinorhynchus psuedomydis* (4.0%), and *Schizamphistomoides sp*. (2.3%), with *Camallanus sp., Spiroxys sp.*, and *Telorchis sp.* making up less than 1% of the remaining sample.

The organs with the least worm burden was the urinary bladder (n=23), with 17.6% of turtles infected followed by the lungs (n=10) with 13.7% infected. The only parasite found in the urinary bladder was *Polystomoides coronatum* while the lungs were infected with the digenetic trematodes, *Spirorchis sp*. (60%), *Diarmostorchis sp*. (30%) and *Heronimus sp.* (10%). Only one individual turtle in the sample contained a dual infection of *Heronimus sp.* and *Spirorchis sp*.

Using simple linear regression to examine relationships to carapace length (ẍ=16.76 cm, range 4.4-30.4), we found a significant positive relationship of total parasite species load to carapace length in female turtles (R^2= 0.448, F=21.13, P < 0.001) (Fig. 1), but not in males (R^2= 0.053, F= 0.913, P > 0.05). For individual parasite species, a significant positive relationship of parasite load to carapace length was found for *Neoechinorhynchus psuedomydis* in female turtles (R^2= 0.479, F=14.75, P < 0.001) (Fig. 2) but not in males (R^2=0.093, F=1.89, P = 0.181 *). For N. emydis*, a significant positive relationship occurred in males (R^2=0.661, F=9.75, P < 0.05) (Fig. 3), but not in females (R^2= 0.048, F= 0.919, P = 0.350). Although other parasite species exhibited a positive correlation to carapace length (primarily in female turtles), none of these were significant (P > 0.05). Only *Diarmostorchis sp*. exhibited no correlation between parasite abundance and carapace length.

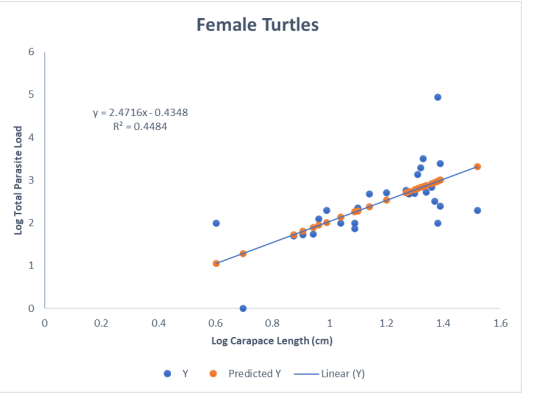

Figure 1. Parasite load plotted against carapace length for female red-slider turtles in Oklahoma (n=30).

Lastly, we compared the results of this study to the Oklahoma study of Everhart by parasite phyla and location in the host (Everhart 1956) (Fig. 4) and by the abundance and percent prevalence of infection (Everhart 1957) (Table 2). Helminth prevalence was considerably higher in this study with several multi-species infections for the red-eared slider, particularly *Neoechinoryhynchus emydis* and *N. psuedemydis* in the small intestine.

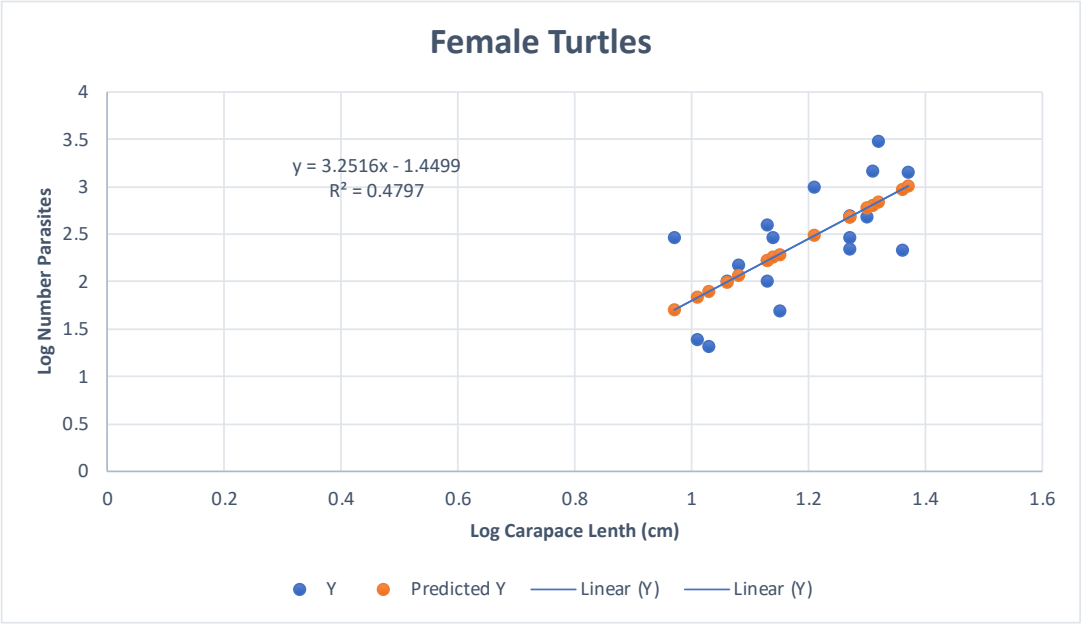

Figure 2. Relationship of parasite load to carapace length for *Neoechinorhynchus psuedomydis* in female turtles.

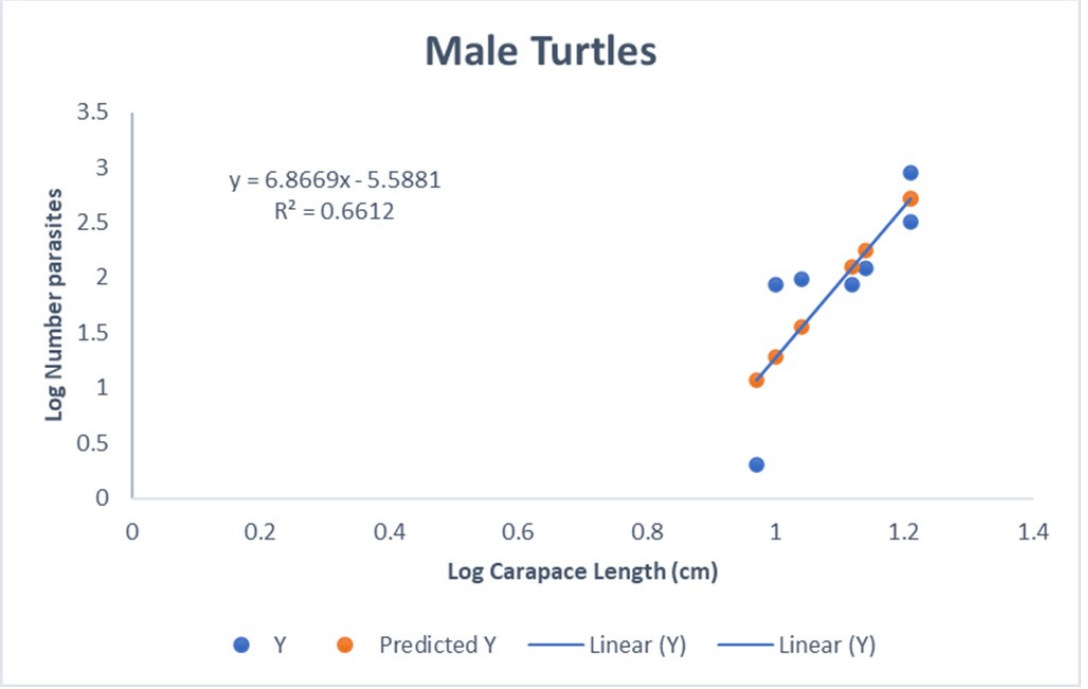

Figure. 3. Relationship of parasite load to carapace length for *Neoechinorhynchus emydis* in male turtles.

Parasite	Everhart 1957			this study		
	abundance (n)	prevalence (%)	ẍ (per host)	abundance (n)	prevalence (%)	ẍ (perhost)
Monogenea:						
Polystomoides coronatum	68	82.6	nr	24	16	3.0
Digenea:						
Telorchis sp.	802	30.6	52.5	456	22	41.5
Spirorchis sp.	28	8.3	5.0	6	10	1.2
Nemotoda:						
Camallanus sp.	1486	74	18.0	3062	92	66.6
Spiroxys sp.	no adults recovered only encysted larvae reported			1278	78	32.0
Acanthocephala:						
N. emydis	377	47.8	nr	5154	63	161.1

nr= not reported

Table 2. Prevalence of same parasites reported by the Oklahoma study of Everhart (1957) (n=23 turtles) compared to the results of this study (n=45 turtles).

Discussion

The prevalence of helminth infection was considerably higher in this study compared to the Oklahoma study of Everhart (1956, 1957). Similarly, the study of Rosen and Marquardt (1978) found infections of four acanthocephalan species of red- eared sliders in Arkansas. These findings do question whether competition occurs between these acanthocephalans and if it does, it may be evident in some of their shared intermediate hosts, particularly in the altering of host phenotype and behavior (Dezfuli et al. 2001, Rauque and Semenas 2011) or by occupying separate microhabitats within either the intermediate or definitive host. Though *N. emydis* was a slightly more frequent infection (63%) compared to *N. psuedemydis* (57%), no significant difference in mean population size of the two species occurred in co-inhabiting hosts. While we did not determine microhabitat differences between these two species when co-inhabiting hosts, we did find that while both species occasionally occurred in the large intestines, only *N. emydis* was found in the stomachs of a few hosts that were co-inhabited. Further studies about competing effects in these acanthocephalan species is needed.

Since most turtles carried a heavy infection of both species of *Neoechinorhynchus*, it's possible that large numbers of aquatic plants, could provide adequate habitat to support a substantial number of intermediate hosts like ostracods or gastropods (Esch et al. 1979). Even though there was no analysis of aquatic plants in this study, they were quite prevalent within most of the habitats where turtles were collected (pers. observation). Also, as turtles mature, their diet tends to shift towards herbivory (Clark and Gibbons 1969, Ernst and Barbour 1972), and this might explain why parasite load correlates to carapace length in females with *N. psuedomydis* and in males with *N. emydis*. If intermediate hosts are more prevalent among aquatic plants, then foraging turtles shifting to herbivory as they mature (Hart 1983), could be exposed to more infected intermediate hosts as they consume plants. The

correlation of parasite load to shell length has been reported for other turtle species as well (e.g., Texas map turtle, *Graptemys versa*, Lindemann and Barger 2005), even though plastron was measured instead of carapace.

Other multi species infections were also observed in the stomachs (*Camallanus sp*. and *Sproxys sp*.) and large intestines (*Spironoura sp*., *N. emydis*, *N. psuedemydis* and *Schizamphistomoides sp*.) and rarely the lungs (one individual host with *Spirorchis sp*. and *Diarmostorchis sp*.) but these were much less common compared to the small intestine. The trematode *Spirorchis sp*. is primarily reported from circulatory organs (Williams 1953, Holliman and Fisher 1968) with some species in the brain (Platt 2000). While lung infections by certain species do occur (Roberts et al. 2016), there are scant reports in the literature for the species *Spirorchis emydis* and *Diarmostorchis* sp. Although the study of Oklahoma red-eared sliders by Everhart (1957) does not report any data for muli-species infections, some of the same species and/or genera were recovered, so its highly likely co-inhabitation of the same organs did occur. Further comparisons to Everhart (1956, 1957) were somewhat difficult since the data was not collected and reported as in this study. However, we were able to make some phyla and species comparisons and there were notable differences in the abundance of acanthocephalans, particularly of organs like the stomach and large intestine, which were not reported in the turtles examined by Everhart (1956, 1957). The present study reports far more nematode infestations in the stomach, small intestine, and large intestine but no infestation of the mouth, esophogus and heart as reported by Everhart (1956, 1957). Are these differences an indication of a shift in parasite prevalence in the host over time, or indicative of a change in the type and/or abundance of intermediate hosts? The answer lies with more intensive studies on red- eared slider enteric parasitism.

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