An Assessment of Leech Parasitism on Semi-aquatic Turtles in the Western Piedmont of North Carolina

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Abstract - In 2005, we assessed the occurrence of leeches on semi-aquatic turtles in nine ponds in the North Carolina Piedmont. *Placobdella parasitica* (smooth turtle leech) was the only parasitic leech found on turtles and was present on turtles from all ponds. Female *Chrysemys picta* (Painted Turtles) were more frequently parasitized than males (females 54.7%, males 40.9%; $p = 0.039$), possibly because they are larger and provide more surface for leech attachment. *Chelydra serpentina* (Snapping Turtles) had the highest leech load of any species (mean = 32.3/turtle), which we attributed to its large size and bottom-dwelling habits. Most leeches were found attached to the underside of marginal scutes or between the plastron and inguinal region. These sites likely offer protection from the environment when a turtle emerges from the water.

Introduction

Leeches are common ectoparasites of many freshwater vertebrates including turtles (Ernst et al. 1994, Sawyer 1986), amphibians (Briggler et al. 2001), and fish (Pearse 1924). In North America, leeches of the genus *Placobdella* are commonly found on turtles (Sawyer 1972, 1986), and several studies have examined aspects of these host-parasite relationships such as parasite loads (Brooks et al. 1990, Hulse and Routman 1982, MacCulloch 1981), seasonal variation in parasite infestations (Ernst 1971, Graham et al. 1997, Koffler et al. 1978), variation in attachment locations (Brooks et al. 1990, Dodd 1988), recolonization rates (Dodd 1988, Ryan and Lambert 2005), and leeches as vectors of hemogregarine blood parasites (Paterson and Desser 1976; Siddall and Desser 1991, 2001).

Most studies of turtle-leech interactions have focused on only one or two species of turtle. Bottom-dwelling species such as *Chelydra serpentina* Linnaeus (Common Snapping Turtle) and the mud and musk turtles (Family: Kinosternidae) generally have higher parasite loads than other semi-aquatic turtles (Brooks et al. 1990, Ernst 1986). Aerially basking species (e.g., emydids) generally have reduced parasite loads, possibly because basking forces leeches to detach to avoid desiccation (Ernst 1971, MacCulloch 1981, McAuliffe 1977).

Studies examining turtle-leech relationships have been primarily conducted in relatively large bodies of water (e.g., lakes and rivers). Previous studies have been conducted at the Qu'Appelle River in Saskatchewan

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(MacCulloch 1981), the Upper Warrior Basin in Alabama (Dodd 1988), the Central Canal in Indianapolis, IN (Ryan and Lambert 2005), and the Drysdale and Isdell Rivers in Australia (Tucker et al. 2005).

In our study, we describe turtle-leech relationships within nine relatively small, isolated golf course and farm ponds in the western Piedmont of North Carolina. Specifically, we (1) describe gender and species-specific differences in frequency of parasitism and leech load, (2) examine relationships between turtle size and leech load, (3) examine relationships between parasitism and turtle body condition, (4) describe variation in parasitism between pond types, and (5) describe leech attachment sites on host turtles.

Methods

We assessed the occurrence of leeches on semi-aquatic turtles as part of a mark-recapture study of turtles at nine man-made ponds in Mecklenburg, Iredell, and Cabarrus counties, NC from 17 April to 28 July 2005. Four ponds were located on golf courses, primarily surrounded by residential neighborhoods and fairways. The other five were farm ponds, which contained varying levels of mixed hardwood-coniferous forest and open field within close proximity. Most of these ponds were historically used to provide water for cattle, but none of our study ponds were part of operating farms. The nine ponds ranged in size from 0.03 to 1.02 ha.

We captured turtles using hoop-net traps (model MHNIA, 2.54-cm mesh, Memphis Net and Twine, TN) baited with opened cans of sardines (replaced every 4–5 days). Ten baited traps were used at each site, and were spaced evenly around each pond. We checked traps every other day for a total of 20 days at each pond.

For each turtle we captured, we counted all leeches and recorded their location on the host. We used 4 categories to assess leech attachment site: (1) on the outside of the carapace, (2) on the outside of the plastron, (3) the anterior body (soft tissues on the head, neck, and front limbs), and (4) the posterior body (soft tissues on the hind limbs, inguinal region, and tail). To prevent cross-contamination or loss of leeches, leeches were counted in the field at the time of capture for all turtles except for Snapping Turtles, which we transported individually back to the lab. We removed turtles from the traps one at a time, and immediately examined each turtle for the presence of leeches. We carefully counted leeches on each turtle, and removed some leeches from small leech clusters in order to increase the accuracy of our count. Each leech was classified as small (< 1 cm), medium (between 1 cm and 2 cm), or large (> 2 cm). The same person was assigned the task of counting and classifying leeches each time in order to maintain consistency in leech counts and classifications. Leech data were only recorded for the initial capture of each turtle; all recaptures were noted and released without returning them to the laboratory. Representative leech specimens were taken from turtles at each study pond, preserved in 95%

ethanol for identification, and deposited in the North Carolina Museum of Natural Sciences. After collecting data in the field, all turtles were brought to the laboratory, where we used digital calipers to measure carapace and plastron length, maximal width, and maximal depth to the nearest 0.1 mm for all turtles except large *Trachemys scripta* Schoepff (Yellowbelly Slider) and Snapping Turtles which we measured to the nearest 1 mm. Large Snapping Turtles, were placed in a canvas bag and measured using a spring scale to the nearest 0.2 kg (the mass of the bag was subtracted from the total mass), and we used a top-loading balance to measure the mass of all other turtles to the nearest 0.1 g. All turtles were given a unique mark by filing notches in their marginal scutes (Gibbons 1968, Sexton 1959). This ensured that we would not double-count leeches on an individual turtle if recaptured. Sex of adult turtles was determined by claw length, shell shape, and tail length for *Chrysemya picta* Schneider (Eastern Painted Turtle), *Pseudemys concinna* LeConte (Eastern River Cooter) and Yellowbelly Slider; shell shape and tail length for *Kinosternon subrubrum* Lacépède (Eastern Mud Turtle) and *Sternotherus odoratus* Latreille (Common Musk Turtle) (Ernst et al. 1994); and by using the formula described by Mosimann and Bider (1960) for Snapping Turtles. We attempted to age each turtle by counting the rings on the plastron and/or carapacial scutes, and used a confidence scale ranging from 0 to 3 to rate the accuracy of the count (Cagle 1946, Sexton 1959). We assumed for all turtles that one new growth ring was added each year (but see Wilson et al. 2003). Many adult turtles could not be aged accurately because the growth rings were not clearly visible; therefore, they were categorized as "old." We processed and returned all turtles to the original capture site within 2–4 days of capture.

Data analysis

We used chi-square tests to determine if there were gender- or species-specific differences in the number of turtles parasitized. To better estimate the potential impact of number and size of leeches, leech loads for each turtle were calculated by multiplying the number of leeches by a size class assigned to each leech. Small leeches were assigned a constant of 1, medium-sized leeches were assigned a 2, and large leeches were given a value of 3. For example, a turtle with one small leech and two medium leeches would have a leech load of 5. This method assumes that larger leeches have a greater impact on the animals than do smaller leeches and, although this analysis only estimates the overall impact, it provides more information than simply counting the number of leeches. We used a Wilcoxon rank-sum test to determine any differences in leech load between genders.

To determine if there were any differences in leech load between pond types or species, we used a two-way analysis of variance (PROC GLM; SAS Version 9.1, SAS Institute 1999, Cary, NC). The high number of zeros (i.e., turtles with no leeches) skewed the data so that tests could not be performed due to violation in normality. Therefore, we used only data for turtles with at least one leech in our analysis. Thus, many of our data points were omitted, so we could only perform these tests on three species (Snapping Turtles, Eastern Painted Turtles, and Eastern Mud Turtles). All leech loads were log-transformed to normalize the dataset before analysis.

We also tested for differences in average leech load between pond types for each species using the Wilcoxon rank-sum test. This test allowed us to use all data points (including turtles with no leeches) to examine the relationship between leech load and pond type for each species of turtle.

We conducted a linear regression analysis to examine the relationship between plastron length and leech load for Eastern Painted Turtles and Yellowbelly Sliders. We used carapace length for Snapping Turtles and Eastern Mud Turtles to examine this relationship because their plastron lengths are highly variable (Lindsay and Dorcas 2001). We also used linear regression to determine if a relationship existed between turtle size and the size of leeches parasitizing them. We performed three linear regressions for each turtle species, one for each leech size class. We used the number of leeches in each size class as the dependent variable and size of the turtle as the independent variable.

To examine the relationship between parasitism and body condition, we used a linear regression with leech load as the independent variable and body condition as the dependent variable. Condition was calculated as the residuals of a linear regression with mass as the dependent variable and plastron length (Eastern Painted Turtles and Yellowbelly Sliders) and carapace length (Snapping Turtles and Eastern Mud Turtles) as the independent variable (Budishak et al. 2006, Lindsay and Dorcas 2001). We used an alpha of 0.05 for all analyses.

Results

We observed leeches on all species captured, which included Eastern Painted Turtles, Yellowbelly Sliders, Snapping Turtles, Eastern River Cooters, Eastern Mud Turtles, and Musk Turtles. *Placobdella parasitica* Say (smooth turtle leech) was the only parasitic leech found, and was present on turtles from all ponds. During the study, we captured 221 Eastern Painted Turtles, of which 47.5% were parasitized by at least one leech. We captured 34 Snapping Turtles, of which 67.6% were parasitized. Of the 37 Yellowbelly Sliders captured, 48.6% bore leeches, and of the 27 Eastern Mud Turtles captured, 63% were parasitized (Table 1).

Female Eastern Painted Turtles were parasitized more frequently than male Eastern Painted Turtles ($\chi^2 = 4.24$, $p = 0.039$) and had higher mean leech loads than males (Wilcoxon rank-sum; $p < 0.01$). No significant sex-specific differences in frequency of parasitism or leech loads were found among the other three species: Snapping Turtles ($\chi^2 = 0.13$, $p = 0.71$), Yellowbelly Sliders ($\chi^2 = 0.24$, $p = 0.62$), and Eastern Mud Turtles ($\chi^2 = 0.02$, $p = 0.88$).

We found Snapping Turtles to have a greater mean leech load than any other species of turtle captured in the study (Fig. 1; two-factor ANOVA: F = 3.9, df = 147, p = 0.02). Snapping Turtles were parasitized more frequently than Eastern Painted Turtles (χ^2 = 4.78, p = 0.02). Snapping Turtles and Eastern Mud Turtles

Table 1. Prevalence of leeches on four species of semi-aquatic turtles in nine ponds located in Mecklenburg, Cabarrus, and Iredell counties, NC. Leech loads were calculated by multiplying the number of leeches by the size class of each leech. Small leeches (< 1 cm) were assigned a size class of 1, medium-sized leeches (between 1 cm and 2 cm) were assigned a 2, and large leeches (> 2 cm) were given a value of 3.

Species	n	Number parasitized	Percent parasitized	Mean leech load	Range (leech load)
Eastern Painted Turtles	221	105	47.5	3.84 ± 0.59	
Male	115	47	40.9	2.97 ± 0.77	0–68
Female	106	58	54.7	4.78 ± 0.91	0–71
Snapping Turtles	34	23	67.6	32.26 ± 14.47	
Male	17	12	70.6	21.18 ± 8.40	0–129
Female	17	11	64.7	43.35 ± 27.88[A]	0–468
Yellowbelly Sliders	37	18	48.6	7.11 ± 3.06	
Male	19	10	52.6	6.05 ± 2.79	0–50
Female	18	8	44.4	8.22 ± 5.65	0–102
Eastern Mud Turtles	27	17	63.0	6.33 ± 2.52	
Male	14	9	64.3	9.64 ± 4.64	0–66
Female	13	8	61.5	2.77 ± 1.21	0–16

[A]Leech load was 16.81 ± 9.1 when we excluded an outlier value of 468.

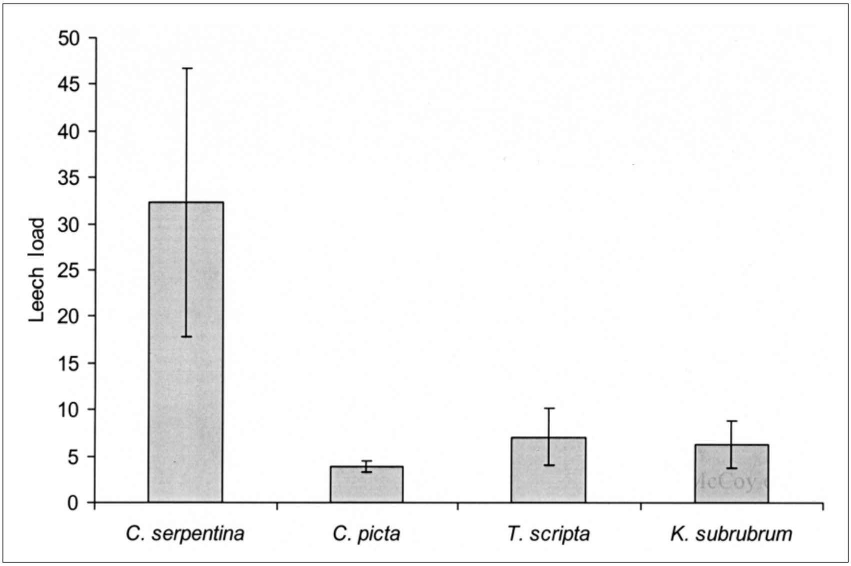

Figure 1. Mean leech loads for *C. serpentina* (Snapping Turtles), *C. picta* (Eastern Painted Turtles), *T. scripta* (Yellowbelly Sliders), and *K. subrubrum* (Eastern Mud Turtles). Leech loads were calculated by multiplying the number of leeches by the size class of each leech. Error bars represent ± one standard error.

were parasitized at similar frequencies, meaning both species had a similar percentage of individuals with at least one leech ($\chi^2 = 0.15$, $p = 0.70$).

Leech size varied widely from less than 0.5 cm to greater than 4 cm. We found no relationship between leech size and the size of the host turtle ($p > 0.05$ for all analyses). However, our data suggest that larger leeches were more prevalent on larger species of turtles, Snapping Turtles and Yellowbelly Sliders (Table 2). We found no relationship between the turtles' body condition and the leech load (all p-values > 0.19). We found a positive relationship between leech load and plastron length for Eastern Painted Turtles ($R^2 = 0.033$, $p = 0.004$), although the low R^2 value explains little of the variation, possibly rendering this finding biologically meaningless.

Table 2. Number and prevalence of leeches, grouped by size class, on four species of turtles from nine ponds located in Mecklenburg, Cabarrus, and Iredell counties, NC. Small leeches (< 1 cm) were assigned a size class of 1, medium sized leeches (between 1 cm and 2 cm) were assigned a 2, and large leeches (> 2 cm) were given a value of 3.

	Size class 1		Size class 2		Size class 3	
Species	n	#/turtle	n	#/turtle	n	#/turtle
Snapping Turtles (n = 34)	204	6.00	216	6.35	17	0.50
Eastern Painted Turtles (n = 221)	369	1.67	137	0.62	69	0.31
Yellowbelly Sliders (n = 37)	97	2.62	56	1.51	18	0.48
Eastern Mud Turtles (n = 27)	156	5.78	3	0.11	3	0.11

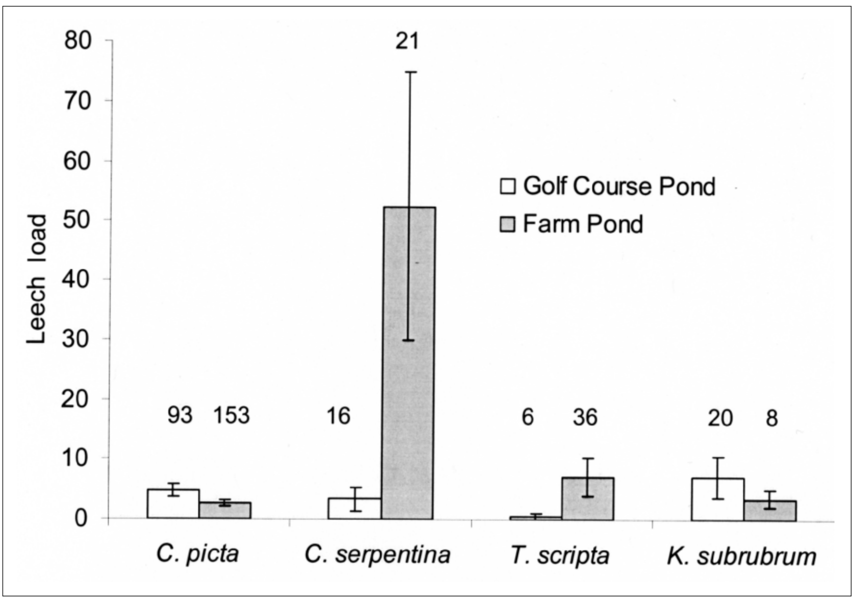

Figure 2. Mean leech loads for *C. picta* (Eastern Painted Turtles),*C. serpentina* (Snapping Turtles), *T. scripta* (Yellowbelly Sliders), and *K. subrubrum* (Eastern Mud Turtles) in golf course ponds and farm ponds. Numbers represent sample size (number of turtles). Leech loads were calculated by multiplying the number of leeches by the size class of each leech. Error bars represent ± one standard error.

There were no overall trends in mean leech load between pond types (two-factor ANOVA: df = 147, F = 0.30, p = 0.58). However, mean leech loads for Snapping Turtles were higher in farm ponds than in golf course ponds (Fig. 2; Wilcoxon rank-sum: $p < 0.001$). Eastern Painted Turtles had higher mean leech loads in golf course ponds than in farm ponds (Fig. 2; Wilcoxon rank-sum: $p = 0.02$).

Leeches attached more frequently on the posterior region of Eastern Painted Turtles and Eastern Mud Turtles, while they attached more frequently on the anterior region of Snapping Turtles. They were found in relatively equal proportions on the anterior and posterior regions of Yellowbelly Sliders. Leeches were found on the carapace of Snapping Turtles more frequently than any other species (Fig. 3).

Discussion

The smooth turtle leech is the most common species of leech found on turtles in the northern United States and Canada (Klemm 1995, Sawyer 1972). In our study, the smooth turtle leech was the only leech found parasitizing turtles, confirming that the species is also well established in the western Piedmont of North Carolina. All turtle species captured were parasitized, although the frequency of parasitism and mean leech load varied considerably among and between species, gender, and pond type.

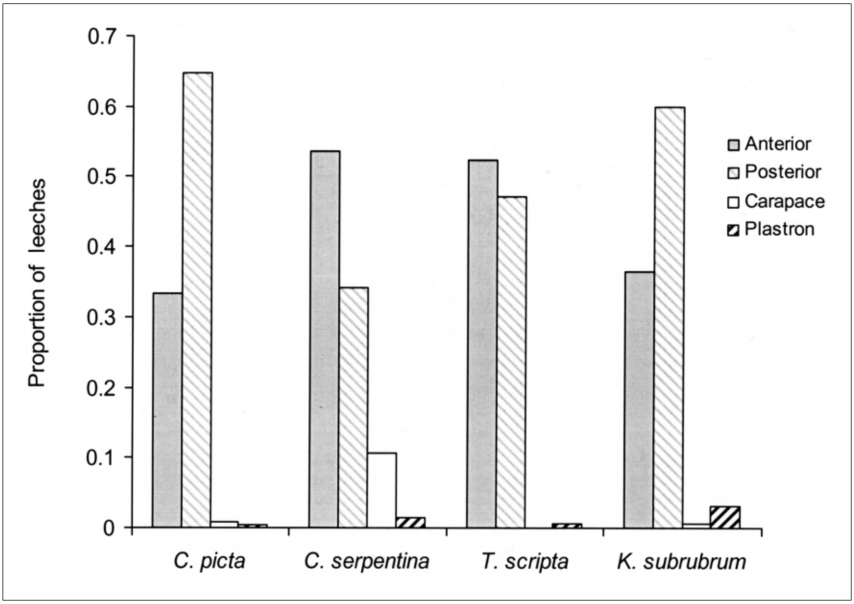

Figure 3. Leech attachment sites for *C. picta* (Eastern Painted Turtles),*C. serpentina* (Snapping Turtles), *T. scripta* (Yellowbelly Sliders), and *K. subrubrum* (Eastern Mud Turtles). Each bar represents the number of leech observations at each region as a proportion of the total number of leeches found on each species.

Gender-specific differences

In our study, the only species to show gender-specific differences in parasitism was the Eastern Painted Turtle, in which females were more frequently parasitized and had higher leech loads than males. Sexual dimorphism is prominent in Eastern Painted Turtles, with females generally larger than males (Ernst et al. 1994). We also found a significant, though weak, relationship between increased plastron length and leech load for Eastern Painted Turtles; therefore, the higher prevalence of leeches on female Eastern Painted Turtles may be due to their larger size. Although there were no significant differences between sexes of other species, male Snapping Turtles had a higher rate of parasitism than female Snapping Turtles. Sexual dimorphism is also prominent in Snapping Turtles, where males are typically larger than females (Ernst et al. 1994). Brooks et al. (1990) reported more leech clusters on male Snapping Turtles than females and attributed it to size differences. Thus, size could be a determining factor in both frequency of leech parasitism and leech loads for Eastern Painted Turtles and Snapping Turtles. Future studies could experimentally examine rates of parasitism between sexes and sizes by removing leeches and examining recolonization rates.

Species-specific differences

Snapping Turtles were parasitized more frequently and had higher mean leech loads than any other species in our study. Brooks et al. (1990) found extremely high rates of leech parasitism on Snapping Turtles in Algonquin Park, ON. Due to their large size and bottom-dwelling habits (Ernst et al. 1994), Snapping Turtles may be more likely than other species to come in contact with smooth turtle leeches, which are notably poor swimmers, spending much of their lives attached to a host or crawling along the pond bottom (Sawyer 1986). Activity level may be a determining factor in turtle-leech relationships. Both Snapping Turtles and Eastern Mud Turtles (bottom-dwellers) had higher frequencies of parasitism than the emydids, Eastern Painted Turtles and Yellowbelly Sliders (active swimmers/baskers).

The frequency of parasitism may also be explained by the "desiccating leech" hypothesis (Ernst 1971, MacCulloch 1981, McAuliffe 1977), which proposes that turtles' basking may force leeches to detach from their hosts to avoid desiccation. Eastern Painted Turtles and Yellowbelly Sliders regularly leave the water to bask on pond edges, logs, and other debris, and in our study they had a lower frequency of parasitism than Snapping Turtles and Eastern Mud Turtles. Our findings correlate with the "desiccating leech" hypothesis, although experimental evidence casts doubt on this hypothesis. Ryan and Lambert (2005) showed that a bottom-dwelling species, the Musk Turtle, acquired more leeches than an aerially basking species, *Graptemys geographica* Lesueur (Common Map Turtle), even when turtles were not allowed to bask. This implies that basking alone does not explain the differences in leech infestations of basking and non-basking species. Combining these findings with evidence that leeches are able to survive after losing up to 92% of the water in their body (Hall 1922), the "desiccating leech" hypothesis may lack some support, but leeches may still choose hosts that are less likely to bask in order to reduce their chances of desiccation (Ryan and Lambert 2005).

Pond types

Combining all species together, we found no overall trend in leech parasitism between farm and golf course ponds. However, there were differences in rates of parasitism between pond types when we examined species individually. Eastern Painted Turtles had higher leech loads in golf courses than in farm ponds. Golf courses generally do not have logs and other debris in their ponds, which would force aerially basking turtles such as Eastern Painted Turtles to bask on the pond edges. Increased presence of humans on and around golf courses may discourage these turtles from basking on the pond edges. Thus, turtles may be forced to spend more time in water, increasing their likelihood of being parasitized.

Leech attachment sites

The attachment locations of smooth turtle leeches on turtles in our study were similar to those found in other studies (Brooks et al. 1990, Dodd 1988, Ernst 1971, Hulse and Routman 1982, Koffler et al. 1978, MacCulloch 1981). The most preferred site of attachment was the posterior region. The majority of leeches in this region were found attached to the underside of the marginals, where they could access the adjacent soft tissue, or between the inguinal region and the plastron. These sites would most likely provide the most protection from desiccation and abrasion when the turtle left the water, as well as protection from predators such as grackles (Vogt 1979), other turtles, and the hosts themselves (Hendricks et al. 1971). Many leeches were found attached in limb sockets where they could also find protection from the environment. Leeches found on the anterior region of the turtles were commonly found on the underside of the marginals, above the head, as well as on the plastron below the head. Similarly, these sites would offer protection from the environment when the turtle was basking. Snapping Turtles had more leeches attached to the carapace and plastron than any other species. Although it is not known if smooth turtle leeches can feed on bony tissues, *Placobdella ornata* (Verrill) (predacious leech) has been observed feeding on bony tissues of turtles (Siddall and Gaffney 2004). The relatively high frequency of occurrence of smooth turtle leeches on the carapace and plastron of Snapping Turtles suggests that smooth turtle leeches can obtain a blood meal from these bony sites.

Several turtle species serve as hosts to leeches. Turtles can be captured easily, and the presence of ectoparasites on each turtle can be readily observed. Studies of leech parasitism on semi-aquatic turtles is an underutilized method of investigating host-parasite relationships, and these turtle-leech relationships can serve as a model system for studying other host-parasite interactions (Dodd 1988, Ernst 1971).

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