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Prevalence and Colonization of *Placobdella* on Two Species of Freshwater Turtles (*Graptemys geographica* and *Sternotherus odoratus*)

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ABSTRACT.—It is generally accepted that bottom-dwelling turtles have a higher ectoparasite load than turtles that bask aerially because of effects of desiccation on ectoparasites, especially with regard to leeches. We compared number of leeches (primarily *Placobdella parasitica*) on field-caught Common Musk Turtles (*Sternotherus odoratus*) and Common Map Turtles (*Graptemys geographica*). The bottom-dwelling species *S. odoratus* had more than 20 times the number of leeches than the aerial-basking species *G. geographica*. We then exposed cleaned (leech-free) turtles to leeches in mesocosms (cattle tanks) to measure the rate of colonization. In this experiment, *S. odoratus* had more than four times the number of leeches as *G. geographica* after 24 h of exposure, even though *G. geographica* were unable to bask under these experimental conditions. We suggest that desiccation threat alone does not explain the species-specific differences in leech loads on aquatic turtles.

Leeches of the genus *Placobdella* are common ectoparasites of North American freshwater turtles (Ernst et al., 1994; Watermolen, 1996). Several species of North American turtle are hosts to the turtle leeches *Placobdella parasitica* and *Placobdella ornata*, which are suspected to be vectors for haemogregrine blood parasites in turtles (McAuliffe, 1977; Siddall and Desser, 2001). However, the nature of the relationship between turtle leeches and their hosts remains unclear and largely unstudied (Brooks et al., 1990). Most previous investigations have been restricted to describing size of parasite loads (MacCulloch, 1981; Hulse and Routman, 1982; Brooks et al., 1990), season-specific parasite load (Ernst, 1971; Koffler et al., 1978; Graham et al., 1997) and locations of infestations on host bodies (Dodd, 1988; Brooks et al., 1990; Saumure and Livingston, 1994).

Bottom-dwelling freshwater turtles, like the Common Musk Turtle (*Sternotherus odoratus*) and the Common Snapping Turtle (*Chelydra serpentina*) generally have higher ectoparasite loads than other aquatic turtles (Ernst, 1986; Brooks et al., 1990). Aerial basking by turtles, especially in the family Emydidae, is believed to reduce ectoparasitsm loads by forcing leeches to disengage in order to avoid desiccation (Ernst, 1971; McAuliffe, 1977; MacCulloch, 1981). Although it is certainly sound reasoning, no data have been collected to test the "desiccating leech" hypothesis. Most previous studies have focused on single species rather than turtle assemblages, and controlled

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experiments investigating the host-parasite dynamics are lacking with regards to *Placobdella* and turtles.

We had two goals in this study. First, we determine the difference in leech infestation between basking and nonbasking turtles species. In the first study, we compared the frequency and intensity of parasitism in field-caught Common Map Turtles (*Graptemys geographica*) and Common Musk Turtle (*S. odoratus*). The former species basks aerially on rocks and logs that border or emerge from the water, whereas the latter species is primarily a bottom-dwelling species. Second, we performed an experiment to determine the rate of colonization by leeches on both turtle species when the opportunity to bask was eliminated. If lower rates of parasitism in basking turtles are caused by desiccation of the leeches, we expect to see higher incidence and intensity of parasitism in *S. odoratus* relative to *G. geographica* in the field study but no difference between the two species in the experimental colonization study.

Materlals and Methods

Field Study.—Our study site was the Central Canal, a 170 year-old human-made canal that flows through urban Indianapolis, Indiana (Conner et al., 2005). We observed *Placobdella* on all six species that make up the turtle assemblage of the Central Canal, which includes Spiny Softshell Turtles (*Apalone spinifera*), Painted Turtles (*Chrysemys picta*), Common Snapping Turtles (*Chelydra serpentina*), and Red-Eared Sliders (*Trachemys scripta*; see Conner et al., 2005). We focus on *S. odoratus* and *G. geographica* because, in addition to the difference in microhabitat use, this pair of species is captured at similar rates at out site (Conner et al., 2005). We collected turtles using aquatic hoop traps (76.3-cm diameter hoops, 30 × 30-cm coated nylon mesh with a funnel at one end and a closed bag at the other) baited with sardines. Turtles were bagged individually at the point of capture and transported to the lab where they were held in isolation to avoid the risk of cross-contamination. We removed and counted all leeches found on adult *S. odoratus* ($N = 71$) and *G. geographica* ($N = 77$) in June and July 2002. We retained these leeches in the lab in canal water for the experimental study (see below). The sex, mass (± 1 g), and straight midline carapace length (± 1 mm) of each turtle was also recorded. As part of an ongoing long-term research project (Conner et al., 2005), we also gave each individual a unique mark (Cagle, 1939) and released the turtles at the point of capture within 24 h. We used two-way analysis of variance (ANOVA) with Type III Sums-of-Squares to determine the effects of species and sex on parasite load. We included log-transformed carapace length as a covariate to account for potential differences caused by body size, and leech counts were natural log-transformed to meet the assumptions of parametric tests.

Experimental Study.—To compare the rates of leech colonization, we placed approximately 650 leeches removed from the field-caught turtles into each of four 1000-liter mesocosms (cattle tanks) located outdoors in the open within 100 m of the Central Canal. The cattle tanks were filled to a depth of approximately 45 cm with aged (> 4 months) tapwater and contained an aged leaf litter substrate. We gave the leeches a 48 h acclimation period, after which we introduced three *S. odoratus* and three *G. geographica* into each tank, for a total of 12 individuals of each species. To limit differences in colonization because of body size, we selected individuals for the experiment that were of comparable size; mean carapace length (log-transformed) of the two species in each tank did not differ (one-way ANOVA: $F_{1,6} = 0.64$, $P = 0.454$). There were no opportunities for turtles to bask aerially in the tanks; the sides of the tanks are sheer and we included no basking platforms.

We recaptured the turtles in the tanks by hand or with a D-frame dipnet 24 h after introduction and counted but did not remove, the leeches on each individual. We returned the turtles to the appropriate tank and repeated the capture/count procedure at 48 h and 72 h after introduction. We terminated the experiment after 72 h and returned the turtles to their approximate point of capture in the canal. Data were analyzed using a repeated-measures ANOVA design with Type III Sums-of-Squares looking at the number of leeches (natural-log transformed) as a function of species and amount of exposure (24, 48, and 72 h). Because there was no difference in body size between individuals in our experiment (see above), we did not use carapace length as a covariate. Means are given ± 1 SE.

Results

In our field study, 98.6% of the *S. odoratus* (70 of 71 individuals) captured had at least one leech, whereas only 50.6% of *G. geographica* (39 of 77 individuals) were infested. The majority of the leeches were *P. parasitica*, with some *P. ornata* present although we did not attempt to quantify the number of each leech species. The incidence of parasitism was not only higher in *S. odoratus*, but they also had a significantly greater number of leeches than did *G. geographica*, independent of body size (species effect: $F_{1,102} = 110.39$, $P < 0.0001$; carapace length [covariate]: $F_{1,102} = 3.57$, $P = 0.062$), and sex did not affect the degree of parasitism in either species (sex effect: $F_{1,102} = 0.11$, $P = 0.741$; sex-by-species interaction: $F_{1,102} = 0.22$, $P = 0.639$; Fig. 1). On average, *S. odoratus* (36.75 ± 4.86) had more than 20 times the number of leeches than *G. geographica* (1.65 ± 0.52). Although more *G. geographica* females (56.8%) were infested with leeches than males (41.2%), the incidence of infestation was not significantly different from parity ($\chi^2 = 0.90$, df = 1, $P = 0.36$).

In our experimental study, we found that leeches colonized all "clean" turtles in the cattle tanks after 24 h of exposure, but the extent of infestation differed significantly between the two species ($F_{1,18} = 92.13$, $P < 0.0001$), with *S. odoratus* having more leeches on average (Fig. 2), a result consistent with our field study observations. The length of exposure had no effect on the number of leeches per turtle for either species ($F_{2,18} = 0.64$; $P = 0.64$; Fig. 2). Likewise, the interaction between time of exposure and species was not significant ($F_{2,18} = 1.09$, $P = 0.36$).

Discussion

Although turtles are known hosts of *Placobdella* (reviewed by Watermolen, 1996), the colonization of turtles by leeches has not been well studied. Dodd (1988) conducted a field experiment where he removed leeches

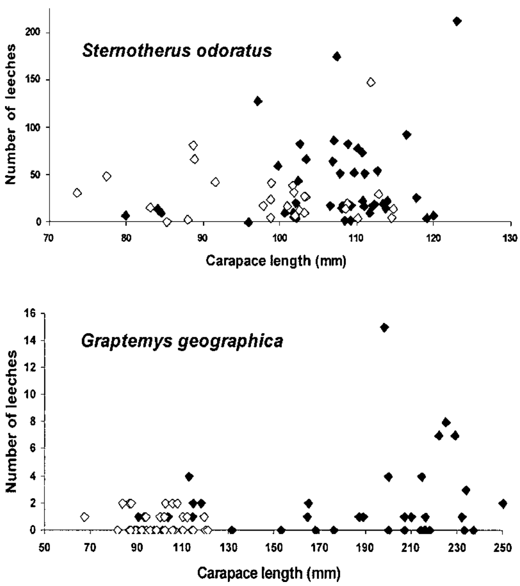

FIG. 1. Relationship between carapace length and the number of leeches in field-caught *Sternotherus odoratus* and *Graptemys geographica*. Open symbols represent males; closed symbols represent females. One outlier (an individual with 36 leeches) has been omitted from the *G. geographica* plot to preserve proper scale.

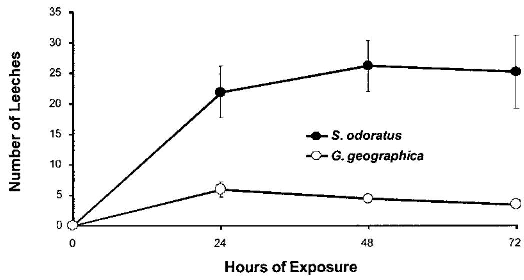

FIG. 2. Colonization of *Sternotherus odoratus* and *Graptemys geographica* by leeches in cattle tanks. Symbols represent means (± 1 SE).

from marked Flattened Musk Turtles (*Sternotherus depressus*), released them and recaptured the turtles to calculate the recolonization rate for individuals (total number of leeches on a recaptured turtle/the number of days between captures). He found (re)colonization rates varied between 0 and 5 leeches/day and that there was no clear relationship between the number of leeches prior to "cleaning" and the number of leeches on the subsequent recapture (Dodd 1988). Aside from a few anecdotal observations (Saumure and Bider, 1996; Saumure and Carter, 1998), Dodd (1988) provides the only quantitative data regarding the rate of leech colonization in nature.

We found higher colonization rates in our experiment compared to Dodd (1988), and at least two factors may be responsible for this difference. First, the baseline level of leech infestation on turtles is greater at our site than in Dodd (1988). For example, field-caught *S. odoratus* in our study (36.75 leeches/turtle) had a substantially greater leech load than in the seven populations of *S. depressus* (average of 7.9 leeches/turtle, with populations ranging between 1.3 and 33.9 leeches/turtle). More important, Dodd's colonization estimates are dependent upon the interval between first and second captures. In our experiment, we assayed the turtles 24, 48, and 72 h after introduction, and the overall number of leeches did not vary significantly between the assays. We obtain different estimates of colonization rates depending on which assay period we use. For example, we would estimate colonization rates of 21.38, 13.09, and 8.38 leeches $\times$ d^{-1} for *S. odoroatus* using the 24, 48, and 72 h assays, respectively. Likewise, our estimates of colonization rate for *G. geographica* would be 5.92, 2.15, and 1.14 leeches/day for when using the 24, 48, and 72 h assays. Dodd (1988) trapped each site in his study at 14-day intervals, meaning that the colonization rates are averaged over the entire 14-day period (or greater if multiple visits to a site were required for a recapture) and assumes a linear colonization rate. Thus for Dodd's population the actual colonization rate may be several times higher than the maximum reported rate of five leeches/day. Our results show that colonization can be very rapid, with host saturation apparently reached within a very short period of exposure. Because of our mesocosms had an abundance of leeches the plateau in colonization is more likely a result of saturation than of running out of leeches.

The results of our field study are consistent with many previous observations where bottom-dwelling turtles have higher ectoparasite loads than do turtles that bask aerially. Unlike many earlier studies, however, our comparison is based on turtle species collected contemporaneously from a common site. Typically, the difference is assumed to be a result of leeches detaching from aerially basking turtles to avoid desiccation. If aerial basking is the proximate mechanism by which basking turtles reduce leech loads, we expect to see similar level of infestation in *S. odoratus* and *G. geographica* in our colonization experiment where we eliminated the opportunity to bask aerially. The bottom-dwelling species (*S. odoratus*) was colonized by leeches at a greater rate than the aerially basking species (*G. geographica*). Based on these results, we suggest that aerial basking alone does not explain the differences in leech loads in these species.

We did not quantitatively monitor the activity level of turtles in the cattle tanks, but our anecdotal observations were that *G. geographica* were somewhat more active (as evidenced by the appearance of individuals at the surface of the water) than *S. odoratus* during the experimental exposure periods. It may be that the lower activity of *S. odoratus* led to longer period of contact with the substrate where the majority of the leeches are presumed to have resided during the experiment. Likewise, leeches have been found in the gut contents of both species (unpubl. data), and, thus, we can rule out that differences we observed were caused by higher cleaning and/or feeding rates of *G. geographica* relative to *S. odoratus*. Kinosternids have smaller plastrons than emydids, and differences in the relative amount of exposed soft tissue may in part explain differences in colonization and overall leech loads. We did not measure this variable in our study since we were not as concerned with the site of attachment (see Dodd, 1988; Brooks et al., 1990) but rather the total leech loads. Future studies will be required to determine whether the amount of exposed soft tissue on species with reduced plastrons, such as *C. serpentina* in addition to the kinosternids, influences colonization rates.

Other (nonexperimental) data also fail to support the "desiccating leech" hypothesis. Brooks et al. (1990) reported the highest level of *Placobdella* infestation on any turtles published in a population of *C. serpentina* from southeastern Ontario that is noted for aerial basking (Obbard and Brooks, 1979), a behavior relatively uncommon for the species. Moreover, in this population male *C. serpentina* had higher numbers of leech clusters than females (Brooks et al., 1990), despite the fact that males basked with a higher frequency (Obbard and Brooks, 1979). Leeches may be considerably more resistant to desiccation than is commonly assumed (Hall, 1922). Vogt (1979) noted anecdotally that he had found live leeches still attached to a *Graptemys* (sp.) after keeping it out of water for four days. In addition, Vogt (1979) observed a common grackle (*Quiscalus quiscula*) feeding on leeches attached to a basking *Graptemys* sp. Although the "desiccating leech" hypothesis lacks support at a proximate level, it may operate at an ultimate level. That is to say, rather than basking turtles having a lower level of infestation because leeches detach when threatened with desiccation, leeches of the genus *Placobdella* may preferentially select hosts that are less likely to bask and, thereby, are exposed to a lower threat of desiccation in the first place. Host choice experiments would be an effective means for testing this hypothesis, an evolutionary alternative to the "desiccating leech" hypothesis, which to this point enjoys only circumstantial support. Additional studies on the habitat choice of unattached leeches are also required to resolve species-specific differences in the parasite-host relationship of *Placobdella* and freshwater turtles.

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