Microhabitat choice as a function of ectoparasitism: basking behavior of *Chrysemys picta bellii* in the presence of *Placobdella* spp.

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ABSTRACT

The “desiccating leech” hypothesis proposes that differences in ectoparasite load between basking and bottom-dwelling freshwater turtle species occur because leeches abandon hosts that spend lengthy periods of time basking out of water and exposed to the sun. However, recent research has put this hypothesis under scrutiny, instead suggesting that leeches have simply evolved to avoid certain turtle species entirely based on the more favorable host environment provided by bottom-dwelling turtles. In order to further examine the validity of the “desiccating leech” hypothesis, the influence of *Placobdella* spp. leeches on the basking behavior of Western painted turtles (*Chrysemys picta bellii*) was investigated through a series of 3-day basking trials. Turtles were randomly assigned to three different treatment tanks: control, *Placobdella* spp., or *Macrobdella decora* (inert organism control). Results of an ANOVA indicated that no significant differences in basking behavior between treatments were observed over the course of the study. Baseline ectoparasite load for each turtle was also taken into consideration. Results of a linear regression analysis complemented the comparison across treatments; no significant relationship existed between baseline ectoparasite load and basking habits. These results support the idea that *Placobdella* spp. have instead evolved to selectively parasitize bottom-dwelling turtle species, regardless of individual basking behaviors.

INTRODUCTION

Basking is a commonly observed behavior in a variety of freshwater turtle species. While basking serves the primary purpose of thermoregulation in ectothermic turtles (Boyer 1965; Ernst 1972; Lefevre and Brooks 1995), it may also have secondary benefits. Basking is not only important in the maintenance of body temperature within a functional range, it is also thought to facilitate increases in metabolic and digestive rates (Moll and Legler 1971; Parmenter 1980;

Hammond et al. 1998; Avery et al. 1993) and promote follicular egg development in females (Vogt 1980; Lefevre and Brooks 1995; Krawchuk and Brooks 1998; Carrière et al. 2008). In addition, basking may be an integral component of skin and shell maintenance by aiding in the periodic shedding of carapacial scutes (Cagle 1950; Boyer 1965; Auth 1975; Shealy 1976).

Of interest to this study is the idea that frequent basking also promotes a reduction in parasite load, known as the "desiccating leech" hypothesis. A variety of North American freshwater turtle species harbor ectoparasites in the form of leeches, most notably the leech species *Placobdella parasitica* and *P. ornata* (Brooks et al. 1990; Siddall and Gaffney 2004; McCoy et al. 2007). The "desiccating leech" hypothesis proposes that turtles with a greater propensity to bask will have a lower leech prevalence due to lengthy periods of time out of water and exposure to the sun, forcing aquatic leeches to abandon their current host (Cagle 1950; Boyer 1965; Ernst 1971; Shealy 1976; McAuliffe 1977; Koffler et al. 1978; McCoy et al. 2007). It has been shown that ectoparasite load is significantly greater in bottom-dwelling turtle species such as the snapping turtle (*Chelydra serpentina*) as compared to basking species such as the painted turtle (*Chrysemys picta*) (McAuliffe 1977; Ryan and Lambert 2005; McCoy et al. 2007; Readel et al. 2008).

However, there is significant doubt to whether this hypothesis is the true explanation for differences in leech load observed between turtle species with contrasting microhabitat choices. MacCulloch (1981) reported no significant difference in leech load between turtles caught while basking and turtles caught in the water, although the "desiccating leech" hypothesis would predict that turtles caught in the water would harbor more leeches. Another study also noted a high leech prevalence in a *C. Serpentina* population that had an unusual tendency to bask (Brooks et al. 1990).

Ryan and Lambert (2005) conducted a colonization experiment in which turtles of both a basking species (map turtles, *Graptemys geographica*) and a bottom-dwelling species (musk turtles, *Sternotherus odoratus*) were collected, cleaned of leeches, and placed in a common tank containing leeches but no basking sites. Initial data on leech prevalence was collected on the wild-caught turtles, showing that *S. odoratus* harbored greater than twenty times the amount of leeches than *G. geographica*. Despite the lack of basking sites under experimental conditions, musk turtles were still colonized by leeches at four times the rate of map turtles, indicating that basking is not the sole factor determining ectoparasite load (Ryan and Lambert 2005).

Leech load may not be a function of basking habits, but rather of bottom-dwelling habits (Ryan and Lambert 2005). Turtle species known to have larger leech loads, such as *C. serpentina*, often spend much of their time near bottom substrates where leeches reside when not attached to a host (Sawyer 1986). This explanation may not be adequate either, however. Readel et al. (2008) found low relative leech prevalence in the spiny softshell turtle (*Apalone spinifera*) and *S. odoratus* in comparison to *C. serpentina* despite all three species sharing a common environment in close proximity to the substrate.

In 2005, Ryan and Lambert suggested an ultimate explanation for leeches' aversion to certain species: leeches have evolved to avoid particular turtle species based on their tendency to bask. Rather than abandoning hosts when threatened with desiccation, leeches simply bypass those specific hosts altogether.

A multitude of studies have examined the "desiccating leech" hypothesis with an interspecific perspective, but few have examined the presence of leeches as potentially correlated with individual basking responses within a single species. If the "desiccating leech" hypothesis is true, then one would expect individual basking behavior to vary in response to the presence of leeches in both an intraspecific and interspecific manner. If the ultimate explanation were true, then one would not expect to see differences in basking behavior depending on the presence or absence of leeches. *Placobdella* leeches would have evolved an aversion to entire species, disregarding individual basking habits within that species. In addition, turtles would not exhibit increased basking behaviors in order to rid themselves of leeches.

This study tested the “desiccating leech” hypothesis in an intraspecific manner by looking at leech loads in Western painted turtles. The Western painted turtle (*Chrysemys picta bellii*) is an ideal species with which to test this parasite-host relationship because it is both a basking species (Boyer 1965), as well as one that harbors *Placobdella* leeches, although in notably lower numbers than bottom-dwelling species (Ernst 1971; MacCulloch 1981; Krawchuk 1997; McCoy et al. 2007; Readel et al. 2008). I predicted that there would not be a significant relationship between leech presence and basking behavior in painted turtles, supporting the ultimate explanation for selective leech parasitism in freshwater turtles rather than the “desiccating leech” hypothesis. Learning more about these parasite-host dynamics allows for a greater understanding of the evolutionary process of ectoparasitism in freshwater turtles.

METHODS

This study was conducted over a 3-week period during July 2013 at the University of Notre Dame Environmental Research Center (UNDERC) on the border of Northern Wisconsin and the Upper Peninsula of Michigan.

Western painted turtles were caught using basking traps, hoop nets, and by hand. Captured turtles were measured (curved carapace length and width, plastron length, and depth) as well as sexed based on foreclaw length and distance between cloaca and carapace (Bayless 1975; Lefevre and Brooks 1995; McCoy et al. 2007). Because the study occurred during nesting season, weight was not recorded as some females were likely gravid. Juvenile turtles were identified as those without male secondary sex characteristics and a carapace length less than 89 mm (Lefevre and Brooks 1995) and were not used in this study.

Turtles were cleaned of large leeches, after which a baseline ectoparasite load was determined for each turtle due to the presence of very small (approximately 1 cm or less) leeches that proved too difficult to remove. For the purpose of identification, each turtle was given an alphanumeric marking on the carapace using nail polish (Snow 1980; Choo and Chou 1984; Gaikhorst 2011). Turtles were fed a diet of Tetra ReptoMin Floating Food Sticks.

Five basking trials were conducted. Three tanks 1.75 m in diameter were filled with fresh lake water (Tenderfoot Lake) to a depth of 30 cm before each trial. A basking platform with approximately 0.12 m^2 of space above the water surface was placed in each tank (Figure 1). A heat lamp (65 watts) was angled approximately 25 cm over each basking platform, and was turned on for the 12-hour duration of each daily basking period. The experimental tank contained 10 large (over 3 cm) *Placobdella* leeches, while an inert control tank contained 10 *Macrobdella decora* leeches and a second control tank contained no additional leeches. North American Freshwater leeches (*Macrobdella decora*) do not primarily target turtles and provided an inert organism control. The entirety of the experimental tanks were enclosed in a tarped off area within the lab so that turtles would not be disturbed from their natural basking tendencies by the presence of people. The tarped enclosure was not entered for the duration of each daily basking period.

For each trial, three turtles were randomly assigned to a treatment. Both leeches and turtles were placed in treatment tanks at 8 AM on the first day of each trial. Trials ran for a period of 3 days, and basking behavior was recorded by video camera for a 12-hour period from approximately 8 AM to 8 PM each day. Basking footage was analyzed at 5-minute intervals, with individual turtles marked as present or absent on the basking platform at each interval. A turtle was considered basking if at least 75% of the carapace was visible out of water (Lefevre and Brooks 1995).

An ANOVA was used to determine whether a significant difference in basking behavior occurred between the three treatment tanks across five replicate trials. A replicate was considered one basking trial of 3 days in length. Basking behavior was quantified as the proportion of times a given turtle was present on the basking platform out of total time intervals checked. Linear regressions were also run to determine whether baseline ectoparasite load or size had an effect on basking frequency. Four different size measurements were used: carapace length (curved), plastron length, width (curved), and depth. Two-sample t-tests were used to compare mean basking proportion between turtles with and without leeches, as well as male and female turtles. All statistical analyses were conducted in SYSTAT 13.

RESULTS

Across five basking trials, no significant differences were observed in basking proportion between turtles in the three different treatment tanks ($F = 0.266$, $df = 2$, $p = 0.771$; Figure 2). In addition, no significant relationship was found between baseline ectoparasite load and basking proportion ($R^2 = 0.05$, $p = 0.139$; Figure 3) or any size parameter and basking proportion (Table 1). No significant difference in mean basking proportion was found between turtles with and without leeches ($df = 43$, $p = 0.722$) or male and female turtles ($df = 43$, $p = 0.459$).

An average of 1 out of 10 *Placobdella* leeches attached to turtles in the *Placobdella* tank at the end of each 3-day trial. No *M. decora* were found attached to turtles at the end of any of the trials. Post-trial, both leech species were often found concealed in crevices between the cinder blocks of basking platforms. Only in the first trial were all 10 leeches of each species recovered. An average of 1.8 *M. decora* leeches went missing per trial. *Placobdella* leeches were missing following only one of the five trials, an average of 0.8 per trial.

DISCUSSION

Based on the results of this study, individual basking behavior does not predict or result from *Placobdella* ectoparasite load. No significant differences in basking proportion were found between treatment tanks, indicating that the presence or absence of *Placobdella* leeches does not have an effect on the propensity to bask in Western painted turtles. In addition, no significant relationship was found between baseline ectoparasite load and basking proportion, nor a significant difference in mean basking proportion between turtles with and without leeches attached pre-trial. As expected, these results do not support the “desiccating leech” hypothesis.

It has been well documented that freshwater turtle species are targeted by *Placobdella* leeches at differing rates (McAuliffe 1977; Ryan and Lambert 2005; McCoy et al. 2007; Readel et al. 2008). Multiple explanations have been proposed for this phenomenon. According to the “desiccating leech” hypothesis, turtles with a greater propensity to bask will have a lower ectoparasite load. However, the results of a recent colonization experiment, in which a lack of basking sites did not deter unequal colonization rates between species, lead to another explanation: *Placobdella* leeches have evolved to selectively parasitize bottom-dwelling turtle species based on their more favorable microhabitat choice (Ryan and Lambert 2005).

The lack of significant differences in basking proportion across treatments supports the ultimate explanation for selective leech parasitism, suggesting that although found in small numbers on *C. picta bellii*, *Placobdella* spp. have largely evolved to avoid these basking hosts in favor of bottom-dwelling species such as *C. serpentina*. This idea is further supported by the behavior of the leeches themselves. With an average of only one *Placobdella* leech attaching to a turtle host over each 3-day period, as well as most leeches exhibiting hiding behavior, it seems that both *Placobdella* spp. and *M. decora* actively avoided turtles. Missing leeches post-trial are also an indication that leeches might have been preyed upon by turtles, especially *M. decora*. Additionally, *Placobdella* leeches not attached to a host are often found in bottom substrates; the hiding behavior exhibited in the tank environment may have therefore resulted from the lack of a soft bottom surface within which to reside (Sawyer 1986).

Even if *Placobdella* leeches were to parasitize *C. picta bellii* at rates similar to species such as *C. serpentina*, an increase in basking might not cause leeches to detach. Many of the leeches collected for this study were found on the shells of *C. picta bellii* and *C. serpentina* that had likely been out of water for several hours preparing to nest. *P. parasitica* has also been proven to resist desiccation. Hall (1922) showed that leeches could withstand up to 92% water loss, while Vogt (1979) noted that turtles remaining out of water for four days still retained tightly bound leeches. No reasoning was given for this observation, although it may be that leeches are able to obtain enough fluid from their host to avoid desiccation during periods out of water.

Although *Placobdella* leeches have the ability to survive lengthy periods out of water, remaining in environments below the surface and in closer proximity to bottom substrates may increase survival and fitness rates. As for the discrepancies in leech colonization between *C. serpentina* and other bottom-dwelling turtle species such as *A. spinifera* and *S. odoratus* found by Readel et al. in 2008, perhaps *C. serpentina* provides a more adequate host environment for *Placobdella* spp. for reasons not yet understood. Future research might focus on the differences in parasitism between these bottom-dwelling species and how they might have originated over an evolutionary timescale. It would also be of interest to examine the interplay between host-parasite and predator-prey relationships that appear to coexist between freshwater turtles and *Placobdella* leeches. At least one case of *C. picta* predation on *Placobdella* spp. has been documented, a proposed feeding symbiosis between *C. picta* and highly parasitized *C.* serpentina (Krawchuk et al. 1997).

More remains to be understood about selective leech parasitism in freshwater turtles and how the evolution of this parasite-host relationship may be a model for the development of other parasitic associations. However, as this study shows, the "desiccating leech" hypothesis is likely not valid, and Western painted turtles do not exhibit a behavioral basking response to the presence of *Placobdella* leeches.

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TABLES & FIGURES

	Carapace Length (Curved)	Plastron Length	Width (Curved)	Depth
p-value	0.817	0.645	0.333	0.313
R^2	0.001	0.005	0.022	0.024

Table 1. Relevant statistical values for linear regressions testing the correlation between basking proportion and four different size parameters; no relationships were significant.

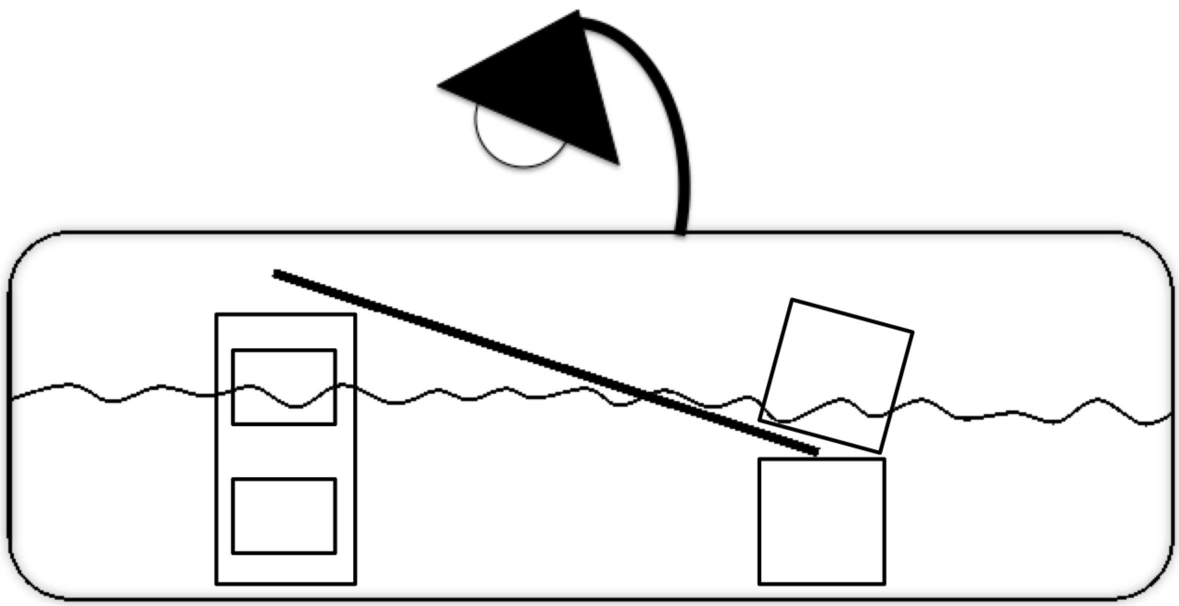

Figure 1. A schematic of the experimental basking platform setup in each treatment tank. Basking platforms were composed of a flat wooden surface secured by three cinder blocks.

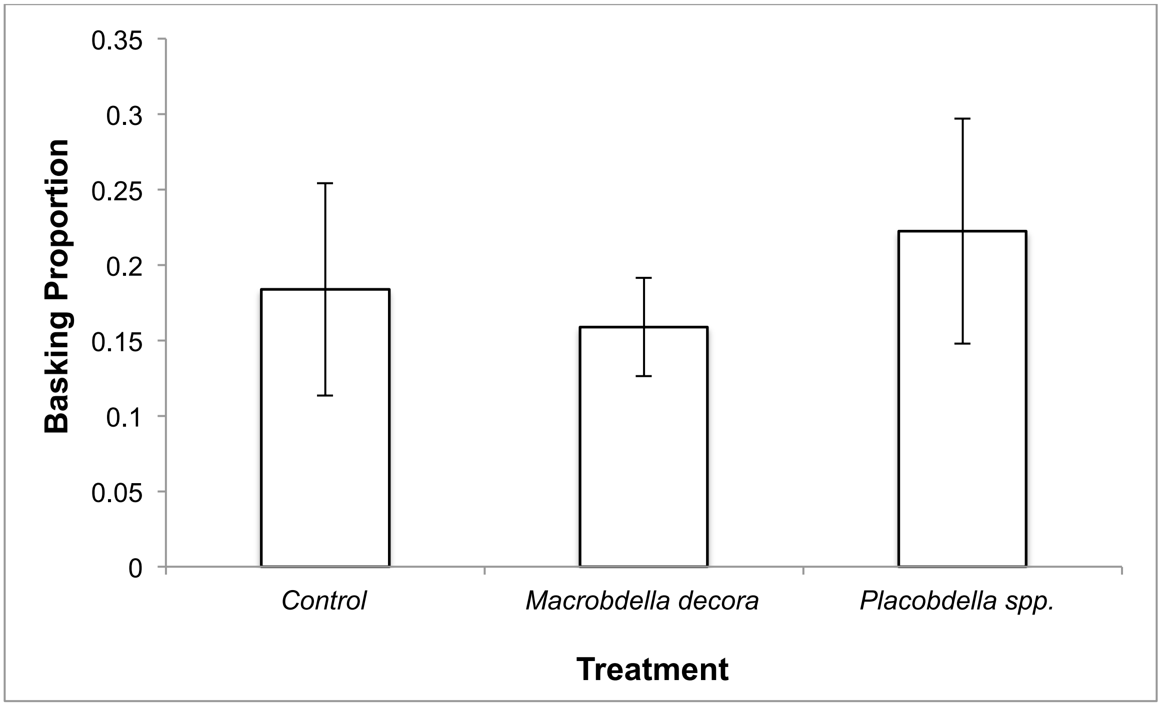

Figure 2. Overall mean basking proportion by treatment with standard error bars.

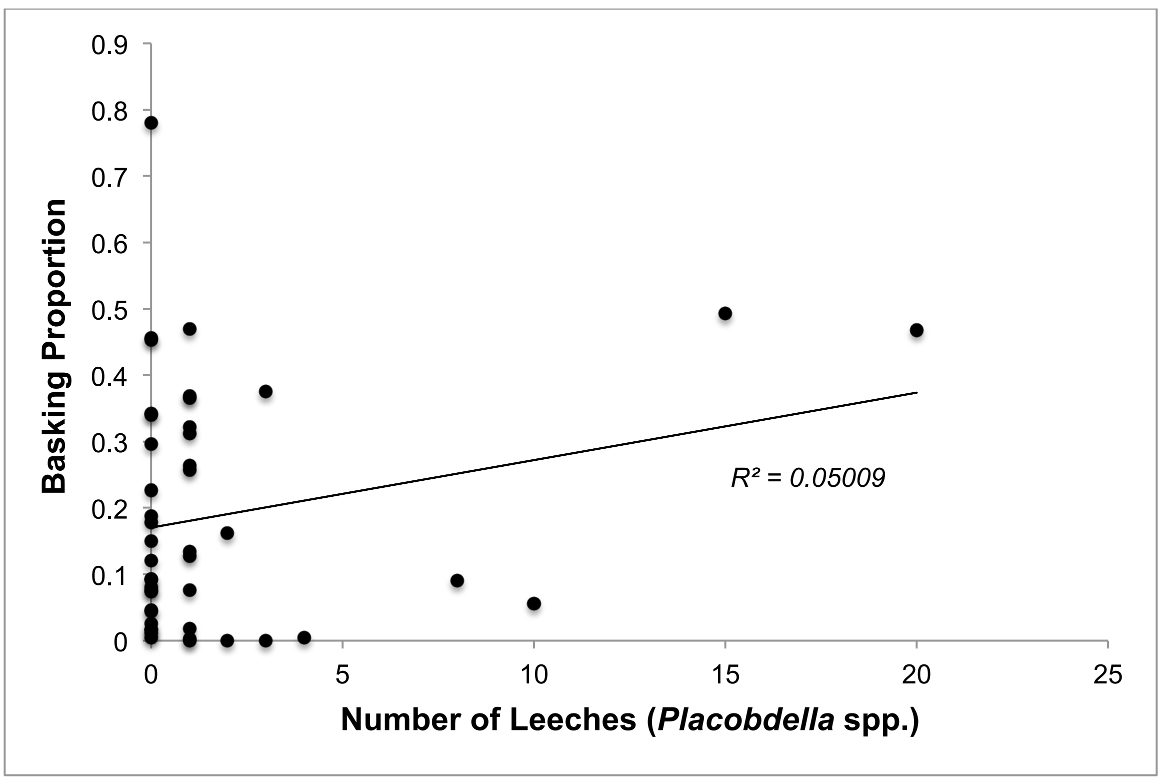

Figure 3. Linear regression between baseline ectoparasite load (*Placobdella* spp.) and basking behavior.

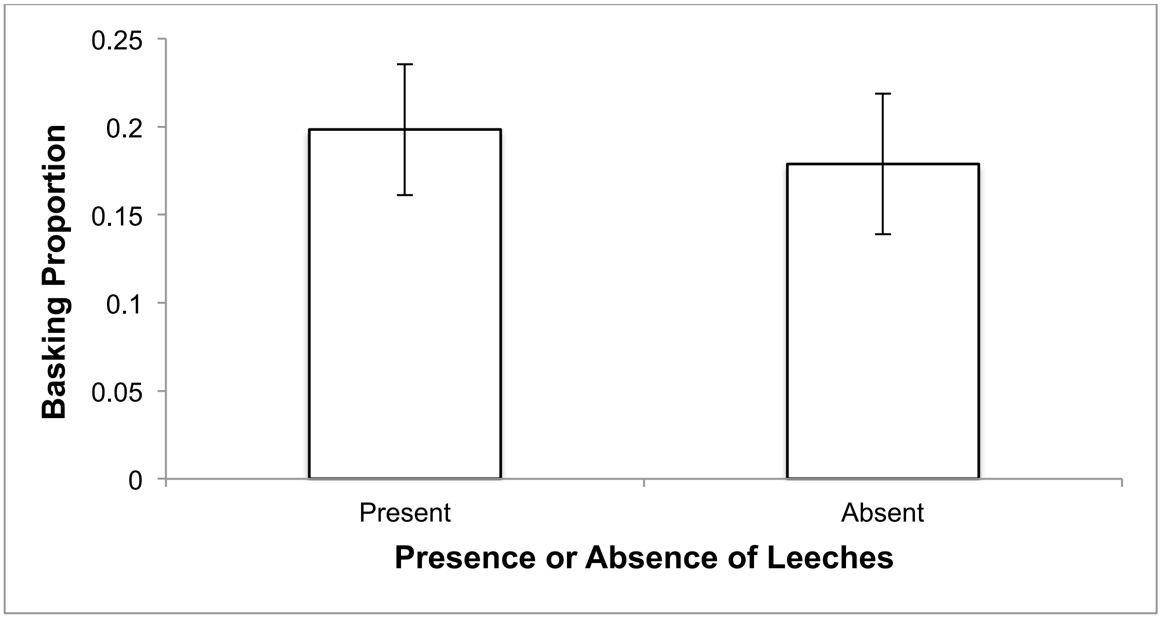

Figure 4. Average basking proportion by presence or absence of baseline ectoparasite load (*Placobdella* spp.) with standard error bars.