Daily activity patterns of movement and refuge use in *Triatoma gerstaeckeri* and *Rhodnius prolixus* (Hemiptera: Reduviidae), vectors of the Chagas disease parasite

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A B S T R A C T

Transmission risk for vector borne disease is greatest during periods of heightened vector activity. Triatomine bugs (Hemiptera: Reduviidae), which transmit *Trypanosoma cruzi*, the causative agent of Chagas disease, are generally considered nocturnal, but their patterns of activity in controlled settings have rarely been studied. We quantified activity patterns across a 24-hour period in nymphs of two triatomine species: (1) *Triatoma gerstaeckeri* Stål, a North American triatomine which is closely associated with sylvatic and peridomestic hosts, and (2) *Rhodnius prolixus* Stål, a Central and South American triatomine that thrives in the domestic environment. *T. gerstaeckeri* showed activity throughout the night, with peaks around midnight, 3:00, and again around dawn. Refuge use was highest in the pre-dawn hours then again after the dawn activity peak. We hypothesize that the dawn activity peak may represent a period of host seeking for this sylvatic species that targets nocturnal hosts returning to their home at dawn. In contrast, *R. prolixus* displayed high activity near 23:00 and again around noon. This study provides a baseline understanding of diel activity for future behavioral experiments and identifies periods of time that may represent the greatest risk of host exposure to triatomines and *T. cruzi*.

1. Introduction

Animals have predictable temporal periods of activity which respond to extrinsic factors such as light intensity, temperature, or humidity, as well as intrinsic factors such as hunger, age, or social rank (e.g. Alanärä et al., 2001; Barrozo et al., 2004). Activity rhythms coordinate behaviors to place them in the optimal context, for example, when predation risk is low and foraging opportunities are plentiful (Monterroso et al., 2013; Sharma, 2003). The partitioning of activity into distinct periods may also reduce the effects of competition, both among species (e.g. Pianka, 1969) and within (e.g. Alanärä et al., 2001).

Temporal partitioning may occur annually, seasonally and over the course of a 24-hour period. Establishing the patterns of daily activity for study organisms may improve the success of animal rearing and behavioral studies (Lorenzo and Lazzari, 1998). For pest species, information about the timing of activity might also be exploited to target control efforts (Fontán and Zerba, 1992) or when preventing colonization of new environments (Lazzari and Lorenzo, 2009). From a human and animal health perspective, the daily activity patterns of arthropod vectors of infectious agents can influence disease transmission dynamics (Sonenshine and Mather, 1994); knowing when vectors are most active can define periods of risk for humans and other affected animals (Curtis-Robles et al., 2018).

Timing of arthropod vector activity is shaped by the temporal availability of hosts, the activity of potential predators, and by environmental conditions relevant to the survival of the vector (Barrozo et al., 2004). For example, *Triatoma infestans* Klug, which transmits the causative agent of Chagas disease (*Trypanosoma cruzi* Chagas), show a bimodal pattern of nightly locomotor activity. The first peak, which occurs near dusk, is associated with increased attraction to CO_2, a host cue (Barrozo, 2003; Bodin et al., 2008), and higher willingness to feed (Lorenzo and Lazzari, 1998), suggesting that bugs search for hosts in the early-night hours. The second peak, around dawn, involves increased orientation towards aggregation pheromones (Bodin et al., 2008) and use of refuges (Lorenzo and Lazzari, 1998). Many hematophagous arthropods follow this pattern, increasing activity at dusk with a second peak of activity in the pre-dawn hours (reviewed in Barrozo et al., 2004). Increased host-seeking activity when hosts become inactive simultaneously increases the probability of host contact while decreasing the probability of detection.

T. infestans and *Rhodnius prolixus* Stål, the triatomines for which diel

activity patterns are most well characterized, are domiciliated insects—they live in close association with their diurnal human hosts. The majority of the 140 + species in the subfamily Triatominae (Bargues et al., 2017), however, are sylvatic, living in association with wild hosts, and far less is known about their chronobiology. We might expect sylvatic and domestic species to differ in the timing of activity because of differences in the timing of host activity. Humans are generally diurnal, so bugs are more vulnerable to extermination while host searching and feeding during daylight hours. In contrast, many of the mammalian species which serve as hosts for sylvatic triatomines—including woodrats, raccoons, armadillos, opossums, and coyotes (Curtis-Robles et al., 2016; Gorchakov et al., 2016; Hodo and Hamer, 2017; Kjos et al., 2013)—are commonly most active during nighttime hours, leaving nests and burrows to forage at dusk and returning near dawn. Thus, diurnal host-seeking activity on the part of sylvatic triatomines might be beneficial.

While domiciliated triatomines are associated with a significant public health burden of Chagas disease in regions of Latin America where they are not controlled, truly domiciliated triatomines are rare in the U.S. (Curtis-Robles et al., 2017; Klotz et al., 2014). However, homes lacking modern screening, caulking, and weather stripping may be subject to kissing bug intrusions and domiciliation, as is the case in south Texas (Curtis-Robles et al., 2017) and southern Arizona (Klotz et al., 2016). In the absence of domestic transmission, *T. cruzi* is maintained between *Triatoma* spp. vectors and wildlife or domestic animal reservoirs with occasional spill-over to humans and domestic animals in the domestic/peridomestic environment (Hodo and Hamer, 2017). In both humans and domestic animals, *T. cruzi* infection often leads to severe cardiac or digestive disease. Across the Americas, an estimated 5–18 million people are infected with *T. cruzi*, resulting in ~10,000 deaths every year (Stanaway and Roth, 2015; World Health Organization, 2015). Because no vaccination exists for people or animals and anti-parasitic treatments are inadequate, the power to protect human and animal health comes from limiting contact with triatomine insect vectors. Thus, understanding vector behavior is essential for successful vector-borne disease control.

To identify periods of increased risk of *T. cruzi* transmission, we quantified the daily rhythms of locomotor activity and refuge use in *Triatoma gerstaeckeri* Stål nymphs, a species of sylvatic triatomine native to the southern United States and northern Mexico. We simultaneously examined the daily activity patterns of *R. prolixus* nymphs, a domiciliated species widespread in South and Central America. Nymphal triatomines are as capable of transmitting *T. cruzi* as adults, but their behavior is rarely studied (Pavan et al., 2016). Further, we observed insects over a 24-hour period to avoid the assumption of strictly nocturnal behavior.

2. Methods

2.1. Insect collection and rearing

All *T. gerstaeckeri* used in this experiment were 4th or 5th instar F1 offspring of adults wild-caught in South Texas. Adults were received through our citizen science program (Curtis-Robles et al., 2015) or collected by our research team, and were identified to species using Lent and Wygodzinsky (1979). Wild-caught insects were transferred to our USDA-APHIS PPQ-approved BSL2 quarantine facility, maintained at 27–33 °C and 30–60% relative humidity. All insects were initially housed individually in plastic containers lined with filter paper (Whatman Filter Paper, Sigma-Aldrich Inc, Darmstadt, Germany) so that fecal spots could be tested for the presence of *T. cruzi* DNA using molecular methods previously described (Curtis-Robles et al., 2015). Each container was held within a larger plastic tub containing water-impregnated Plaster of Paris to maintain humidity. Individuals were fed defibrinated rabbit or sheep blood (HemoStat Laboratories, Dixon, CA) weekly using Hemotek membrane feeders (Hemotek Ltd, Lancashire,
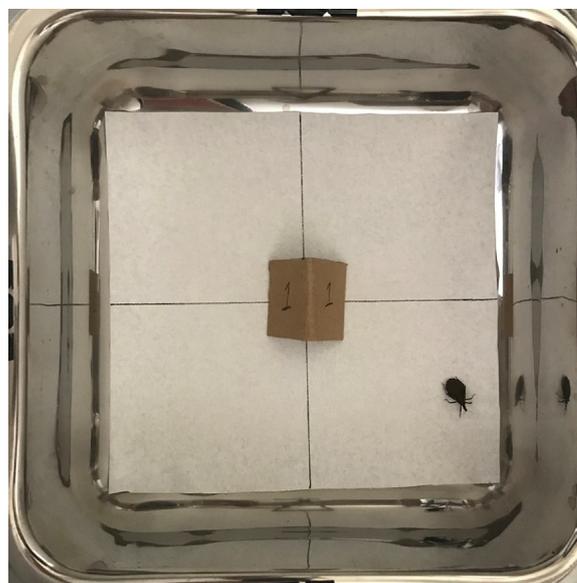

Fig. 1. Stainless steel behavioral arena lined with bench paper. A piece of folded cardboard provided an area of refuge in the center. Crossed lines separate the arena into four equally sized zones.

UK), and paired with opposite-sex adults after *T. cruzi* testing. Resulting F1 nymphs were assumed *T. cruzi* negative because neither transovarial nor coprophagous parasite transmission have ever been observed (Jurberg and Galvão, 2006).

R. prolixus adults, nymphs, and eggs were acquired from the Centers for Disease Control and Prevention through the Biodefense and Emerging Infections Research Resources Repository (BEI Resources, NR-44077 and NR-44076). This strain originated from insect collections in a Columbian domestic environment by Charles B. Beard, but are many generations removed from wild-caught. *R. prolixus* colonies were housed and fed weekly on sheep or rabbit blood as described above. All *R. prolixus* individuals used in this study were 4th or 5th instar.

2.2. Behavioral observations

Behavioral arenas (Fig. 1) were located in a windowed room away from direct sunlight. Each arena was comprised of a 30.48 cm × 30.48 cm × 10.16 cm square stainless steel pan (Hubert Company, Cincinnati, OH) and covered with a 30.48 cm × 30.48 cm × 0.95 cm piece of transparent polycarbonate sheeting (SIBE Automation, Ocala, FL). This arena size is within the range of arena sizes typically used for behavioral experiments with triatomines, and allows room for insect movement (Guarneri et al., 2002; Lorenzo and Lazzari, 1998; Marliére et al., 2015; Minoli et al., 2007; Pavan et al., 2016). Each arena was lined on the bottom with Labmat bench liner (Bel-Art Scienceware, Wayne, NJ) cut to size. The bench paper was crossed by 2 perpendicular lines, creating four equally-sized zones (Fig. 1; Lorenzo and Lazzari, 1998). A 5 cm × 10 cm folded-cardboard refuge was placed in the center of the arena, where the lines crossed. Arenas were cleaned with 10% bleach water and lining paper and refuges were replaced with fresh material between every trial.

All observations occurred between 20 August and 18 September 2017, within the seasonal period of triatomine activity in the southern U.S. (Curtis-Robles et al., 2018). During the observation periods, we recorded room temperatures between 28–32 °C and relative humidities between 31–54% which are within the environmental range supporting the survival and development of triatomines (Guarneri et al., 2002; Luz et al., 1999; Martínez-Ibarra et al., 2008; Roca and Lazzari, 1994; Xavier et al., 2005). Bugs fed to near-repletion were identified and
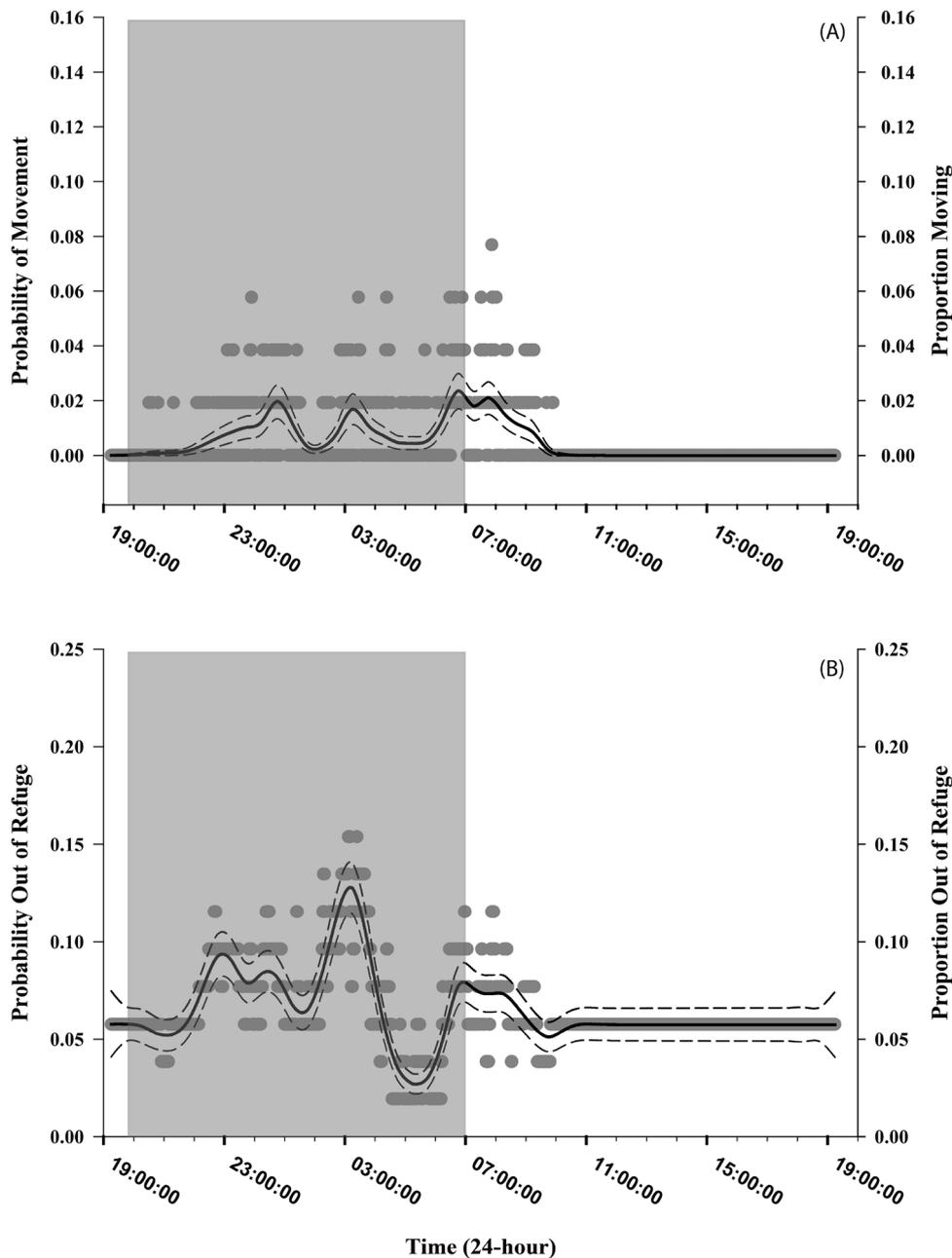
Fig. 2. Model predictions for the probability of locomotor activity (A) and refuge use (B) in *Triatoma gerstaeckeri* nymphs. Grey circles are proportions calculated from raw data. The night phase (sunset-sunrise) is represented by grey shading. Dashed lines are 95% confidence intervals.

starved for 2 weeks to standardize motivation and to promote host-searching behavior (Lorenzo and Lazzari, 1998). In each behavioral arena, a single *T. gerstaeckeri* (n = 52) or *R. prolixus* (n = 8) nymph was released near the center of each arena and allowed to habituate for at least 48 h (Lorenzo and Lazzari, 1998). During habituation and observations, the room was naturally lit and supplemented with low-intensity red light using a 25-watt A19 colored red incandescent lightbulb placed ∼ 2.5 m above the arenas. Triatomines are relatively insensitive to light wavelengths in the dark orange-red range, especially at low intensities (Castillo-Neyra et al., 2015; Reisenman and Lazzari, 2006), and this allowed photographs to be taken at night. Approximately one hour before sunset on the third day we began taking photographs of the arenas at 1 min intervals with Canon™ Rebel XT digital cameras (Canon Inc, Tokyo, Japan) attached to FOTGA intervalometers (Jiataizhe Technology Co., LTD, Shenzhen, China). Photography ended the following day one hour before sunset. Sixteen arenas were photographed

at once (four arenas per camera), comprising a block. Blocks consisted of individuals from both species whenever possible. Consecutive photographs were compared and scored by an observer blind to species for (1) spontaneous locomotion, measured by whether each bug crossed from one zone into another, and (2) location—in refuge or out of refuge. To allow the bugs to recover from potential disturbance caused by starting the cameras, we did not include the first 30 pictures in the comparisons.

2.3. Statistical analysis

To evaluate temporal dynamics of activity, we used Generalized Additive Mixed Models (GAMMs; (Zuur et al., 2009) in R version 3.4.1 using the package 'gamm4' (Wood and Scheipl, 2017). GAMMs relax the assumption of linearity but retain the interpretability of GLMs, allowing nonlinear responses to predictor variables. We constructed
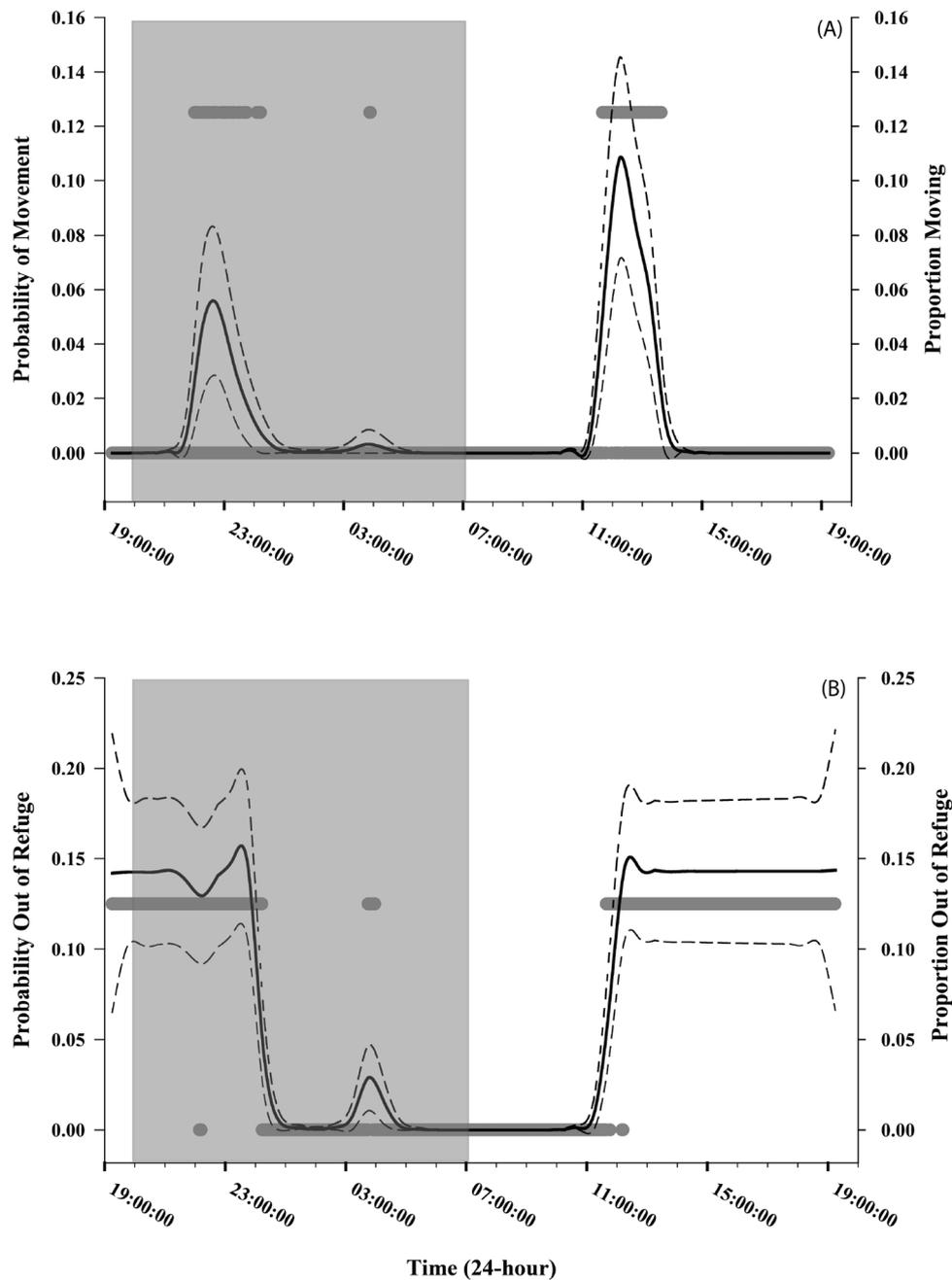
Fig. 3. Model predictions for the probability of locomotor activity (A) and refuge use (B) in *Rhodnius prolixus* nymphs. Grey circles are proportions calculated from raw data. The night phase (sunset-sunrise) is represented by grey shading. Dashed lines are 95% confidence intervals.

models using a binomial link function, using either locomotion or location as response variables and time as a predictor variable. Time was smoothed using the cubic regression spline method (Wood and Scheipl, 2017). Individual ID and date (block) were retained as random effects.

3. Results

We observed 52 *T. gerstaeckeri* and 8 *R. prolixus* nymphs, taking 1440 photographs of each individual. A total of 86,400 observations were made for each response variable (locomotion and location). We saw movement between 0.6% of photographs (0.5% for *T. gerstaeckeri* and 0.9% for *R. prolixus*), and bugs were observed out of refuge in 6.5% of our observations (6.5% for *T. gerstaeckeri* and 6.6% for *R. prolixus*). For *T. gerstaeckeri* and *R. prolixus* respectively, 46% and 13% of the nymphs were associated with at least one movement event and at least

one observation out of refuge.

We observed primarily nocturnal behavior in *Triatoma gerstaeckeri* nymphs (Fig. 2A). This species was active to some degree throughout the night. Peak times of predicted activity occurred between 22:00 and midnight, again at 2:00, and between 6:00 and 8:00, with periods of relative inactivity between. Refuge use followed a similar pattern (Fig. 2B). Nymphs of this species were more likely to be out of refuge between 22:00 and 2:00, then again near dawn. Though 3 individuals (6%) remained out of refuge during daylight hours, they were stationary between 9:30 and dusk. For *Rhodnius prolixus* nymphs, peak movement activity was predicted 3 h after sunset and again around noon (Fig. 3A), with refuge use highest between midnight and noon (Fig. 3B).

4. Discussion

Triatoma gerstaeckeri, a sylvatic triatomine species native to the southern US and northern Mexico, displayed primarily nocturnal locomotory behavior in the nymphal life stage. Activity peaked a few hours after sunset then again around dawn. A previous study found that adult *T. gerstaeckeri* were more likely to be caught at light traps between midnight and 7:00 than between 18:00 and midnight (Pippin, 1970). Further, Flores et al., (2017) observed a peak in captures 2 h past sunset. This latter study was conducted at sunset and lasted only 3.5 h; activity around dawn would have been missed. It may be that nymphs behave differently than adults; future studies will explore this possibility. Additionally, while coprophagous transmission of *T. cruzi* hasn't been shown in any species from the family *Triatominae*, our assumption that the nymphs in this study were free of infection could present the potential for misclassification. Our future studies will address differences in behavior between uninfected and infected individuals. However, it appears from our results, in combination with past observations, that *T. gerstaeckeri* becomes active not at sunset like *T. infestans* (Lazzari, 1992; Lorenzo and Lazzari, 1998; Settembrini, 1984), but later in the evening.

The observed movement peak around dawn for *T. gerstaeckeri* can be attributed in part to refuge use—we also observed a slight decrease in insects found out in the open during this time. However, many hosts found in blood meal analysis of *T. gerstaeckeri* are nocturnal (Kjos et al., 2013), including raccoons and woodrats, and would be returning to their burrows around dawn. Therefore, it is possible that the observed movement around sunrise represents host searching behavior. To our knowledge, the hypothesis of a second period of host searching near dawn in sylvatic triatomines has never been explored. Further, we observed some *T. gerstaeckeri* nymphs out of refuge even during daytime hours. While such behavior in the wild could lead to increased detection by diurnal predators, all exposed insects (each recorded on a different day) were sedentary between 9:30 and sunset. Combined with their drab coloration, remaining stationary may reduce detection by predators that rely on movement and sight to capture prey.

We observed both nighttime and daytime activity peaks in *R. prolixus*. Nymphs of this species (Pavan et al., 2016) and mixed groups of *T. infestans* (Castillo-Neyra et al., 2015) have previously been observed moving around during daylight hours. However, these patterns cannot be confidently interpreted as natural; laboratory colonies are often many generations removed from field-caught, and the selective regimes of lab and field are undoubtedly different. Temperature and humidity fluctuations as well as predation—all factors that shape daily activity rhythms—are greatly reduced or absent in laboratory colonies. Thus, individuals that deviate from "natural" patterns are not pruned from the population. Further, colony insects are often fed during daylight hours, which may entrain daytime meal-seeking. Our sample size for *R. prolixus* nymphs was also rather small (n = 8), deterring strong interpretations of the observed patterns. However, the dawn-dusk rhythms of this species are clearly flexible and behavioral observations of *R. prolixus* are valuable given current restrictions for the containment of this species in Central America.

Characterizing diel activity in disease vectors identifies risky periods when bugs are more likely to be seeking hosts. In the case of *T. gerstaeckeri,* there appears to be a window of high risk between midnight and dawn, affirming the commonly-given recommendation to keep potentially affected animals indoors at night to limit contact with vectors. This study provides a baseline understanding of diel activity for behavioral experiments, including those designed to quantify effects of insecticides or other interventions on locomotor activity and refuge use. We suggest behavioral studies of *T. gerstaeckeri* not focused on diel rhythms be timed to begin two to three hours after initiation of the insects' dark phase to increase likelihood of observations corresponding with peak activity periods. These data might also be useful in parameterizing mathematical models that characterize temporal dynamics of disease transmission (e.g. Yong et al., 2015).

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