

## Daily pattern of locomotor activity of the synanthropic spiders *Loxosceles laeta* and *Scytodes globula*

Rigoberto Solís<sup>1</sup>, Ana Alfaro<sup>1</sup>, Bernardo Segura<sup>1</sup>, Lucila Moreno<sup>2</sup> and Mauricio Canals<sup>3</sup>: <sup>1</sup>Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile; E-mail: rsolis@uchile.cl; <sup>2</sup>Departamento de Zoología. Facultad de Ciencias Naturales y Oceanográficas. Universidad de Concepción; <sup>3</sup>Departamento de Medicina y Programa de Salud Ambiental, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile.

**Abstract.** The locomotor activity of the Chilean recluse spider *Loxosceles laeta* (Nicolet, 1849), and the spitting spider, *Scytodes globula* Nicolet, 1849, were studied in the laboratory under controlled conditions (LD 12:12). In Chile, these are two common synanthropic spiders which share a microhabitat and show extensive thermal niche overlap. Although no systematic studies have been performed, it has been reported that they have nocturnal habits. Here we confirmed strictly nocturnal activity for *S. globula* and mainly nocturnal but with some degree of diurnal activity for the Chilean recluse spider. Also, *S. globula* showed a bimodal pattern of activity while *L. laeta* had a unimodal one. The similarity of the use of space and time make the interaction of these spiders inside homes highly probable.

**Keywords:** Circadian rhythm, niche, overlap, behavior

In Chile, there are two common synanthropic spiders: *Loxosceles laeta* (Nicolet, 1849) and *Scytodes globula* Nicolet, 1849. The former species has been associated with necrotic arachnidism in this country; because of its araneophagic behavior, the latter has been considered as a natural predator of *L. laeta* (Fernandez et al. 2002; Canals et al. 2015a). In spite of its medical and ecological importance, there is scarce information about the behavior of these species. These spiders show similar physiological performance under the same thermal and humidity experimental conditions (Canals et al. 2013, 2015b) which may explain their presence in the same places in homes. However, they differ in trophic exploitation because *S. globula* is mainly an araneophagic spider while *L. laeta* shows a wider prey spectrum (Nentwig 2013). As a consequence, these spiders may use time differently, with *L. laeta* having a less restricted degree of activity than *S. globula*. In addition, preliminary data indicate they also differ in the photic sensibility threshold which produces inhibition of their movements of reaction (unpublished data).

An important amount of documentary evidence indicate that diel rhythms of locomotor activity in spiders are linked to an endogenous rhythm (Cloudsley-Thompson 2013). This activity also appears to be synchronized to the light-dark cycle, with light intensity being an important temporal environmental cue (Cloudsley-Thompson 2000). Because time is an important axis of the niche which may make possible the spatial coexistence of species, this study helps to understand the dynamics of the interaction of two synanthropic species in Chile. Here we report differences in locomotor behavior between the species maintained under an experimental light-dark cycle.

### METHODS

Adult specimens of *S. globula* and *L. laeta* used in this study were caught inside houses, abandoned warehouses and other dark and little-disturbed synanthropic settings. In the laboratory, spiders were kept in plastic 750 ml screw cap bottles with a light-dark cycle of 12:12. The temperature was maintained at

$20 \pm 2^\circ$  C during the stay in the laboratory and the experiments, which in a previous study was found to be the preferred temperature of these species (Alfaro et al. 2013). Throughout the study, the spiders were fed with larval stages of *Tenebrio molitor* twice a month and watered using a spray bottle twice per week.

Locomotor activity of 18 individuals (5 males and 13 females) of *S. globula* and 18 individuals of *L. laeta* (9 males and 9 females) was recorded. Seven days before the start of the activity recordings, spiders were transferred to an arena for habituation. The arena consisted of a white plastic circular container (19.5 cm diameter and 5 cm deep) covered by a glass plate.

The measurements were made inside an experimental chamber (0.6 m wide x 1.3 m long x 1.5 m high), where the same maintenance cycle of light: dark (12 hours light and 12 hours dark) was applied. As a source of light, a 40 W bulb with tungsten filament produced 20 lux intensity. In order not to alter the behavior of individuals during the dark phase of the cycle, an infrared light was used (Canals et al. 1997), and the recordings were made with the “nightshot” function of a SONY HDR-CX 700 digital video camera for infrared lighting for night vision. To our knowledge there is no previous information on the sensitivity of spiders to infrared light, and available evidence describe maximal sensitivities at wavelengths lower than 600 nm (Foelix 2011). In agreement with these observations, we did not detect any behavioral changes in the species we used under IR.

All spiders were recorded in photophase first and then in scotophase. After the diurnal activity was recorded, the L:D cycle was inverted in the experimental chamber in order to match the natural cycle and our work schedule, because the nightshot function needs to be activated manually. The experimental subject was exposed to the new cycle for at least seven days with the new cycle before its nocturnal locomotor activity was recorded. Available evidence indicates that invertebrates are able to synchronize to a new L:D cycle very rapidly (Ortega-Escobar 2002; Mistlberger & Rusak 2005). Thus, locomotor activity during photophase and scotophase

Table 1.—Average of movement events (ME) and distance walked (DW) during 12 hours of light (L) and 12 hours of dark (D) by *Scytodes globula* and *Loxosceles laeta*

	ME (n)		DW (cm)	
	L	D	L	D
<b><i>S. globula</i></b>				
Males	67 ± 18	1009 ± 461	18 ± 6	222 ± 117
Females	35 ± 30	1101 ± 324	9 ± 6	553 ± 231
All individuals	45 ± 21	1092 ± 257	12 ± 6	443 ± 161
Proportion of activity (%)	4.1	95.9	2.6	97.4
<b><i>L. laeta</i></b>				
Males	723 ± 304	1619 ± 540	535 ± 355	845 ± 262
Females	803 ± 281	2441 ± 540	274 ± 389	424 ± 332
All individuals	763 ± 200	2132 ± 355	407 ± 186	635 ± 147
Proportion of activity (%)	26.4	73.6	39	61

of each individual was recorded only for one 12-hour period, the last of the seven days of acclimation to the normal or inverted cycle L:D.

Locomotor activity was measured as the number of movement events (ME) of the spider in the arena during the scotophase and photophase. The criterion for movement was a position shift not less than the body length of the spider. In each phase, the activity was recorded continuously with the camera connected to a Noldus Ethovision system (Noldus Information Technology, Wageningen, The Netherlands) using a sampling rate of one frame per second. Variables measured were number of movement events (ME) and total distance walked (DW) by the spider during recordings. To discern a temporal pattern of activity, the number of movements per hour was plotted both in the light and dark phases. To compare between phases, species and sex, a factorial analysis of variance was performed.

Frequency histograms were constructed for total ME per hour for each species and the overlap resulting of the temporal use of the time axis was calculated using Pianka's index (Pianka 1973):

$$O_{jk} = \frac{\sum_i p_{ij} p_{ik}}{\sqrt{\sum_i p_{ij}^2 \sum_i p_{ik}^2}}$$

$O_{jk}$  is index of overlap (0 to 1) of species "j" and "k", and  $p_{ij}$  is the proportion of use for species "j" in hour "i". In addition, the activity histogram recorded in the night phase was analyzed looking for unimodality vs. multimodality using the Hartigan test (Hartigan & Hartigan 1985).

Table 2.—Multiple comparisons of ME and DW between species in both phases of L:D cycle. Same letters indicate absence of differences.

	ME		DW	
	L	D	L	D
<i>L. laeta</i>	a	b	ad	b
<i>S. globula</i>	c	d	c	d

The data required transformation to  $(\log(x+1))$  in order to satisfy normality and homoscedasticity assumptions. To verify the parametric assumptions, Shapiro-Wilk and Bartlett tests were applied. In addition, an ANOVA for repeated measures was performed with ME.

## RESULTS

The analyses of the number of movement events of the spiders indicated that both species are nocturnal, with significant differences in the total number of movements during the dark and light periods of the cycle ( $F_{1,60} = 50.37$ ,  $P < 0.001$ ); sex differences were not significant ( $F_{1,60} = 2.14$ ,  $P = 0.14$ ). However, differences were found between species ( $F_{1,60} = 28.52$ ,  $P < 0.001$ ) and there was a significant interaction between cycle and species ( $F_{1,60} = 6.57$ ,  $P = 0.012$ ) (Table 1).

A multiple comparison test showed both species being more active during the night phase and *L. laeta* more active than *S. globula* during both phases of the light-dark cycle ( $P < 0.05$ ; Tukey test) (Table 2).

The repeated measures analysis was consistent with the previous analysis and differences between species were detected ( $\Lambda$ -Wilk = 0.569,  $F_{12,49} = 3.1$ ,  $P = 0.003$ ), a L:D cycle effect ( $\Lambda$ -Wilk = 0.499,  $F_{12,49} = 4.1$ ,  $P = 0.0002$ ), absence of sexual differences ( $\Lambda$ -Wilk = 0.788,  $F_{12,49} = 1.1$ ,  $P = 0.38$ ), and interactions ( $P > 0.05$ ) (Fig. 1).

The activity of *S. globula* was bimodal ( $D = 0.0689$ ,  $P = 0.0008$ ), while the activity of *L. laeta* was unimodal ( $D = 0.0714$ ,  $P = 0.1643$ ) (see Fig. 1).

The analysis of distance walked (DW) also showed differences between the two species, with *L. laeta* wandering a significantly greater distance than *S. globula* ( $F_{1,60} = 26.29$ ,  $P << 0.001$ ), with the largest distances traveled during the light phase ( $F_{1,60} = 31.42$ ,  $P << 0.001$ ), without sex differences ( $F_{1,60} = 1.13$ ,  $P = 0.29$ ) or interaction between phases of the L:D cycle or species ( $F_{1,60} = 8.16$ ,  $P = 0.006$ ). Multiple comparisons showed that both species traveled a greater distance in the scotophase, and with *L. laeta* roaming more actively than *S. globula* in both phases (Tables 1, 2).

The value of Pianka's index was  $OJK = 0.739$ , which indicates considerable overlap in the temporal use of the time axis by both species.

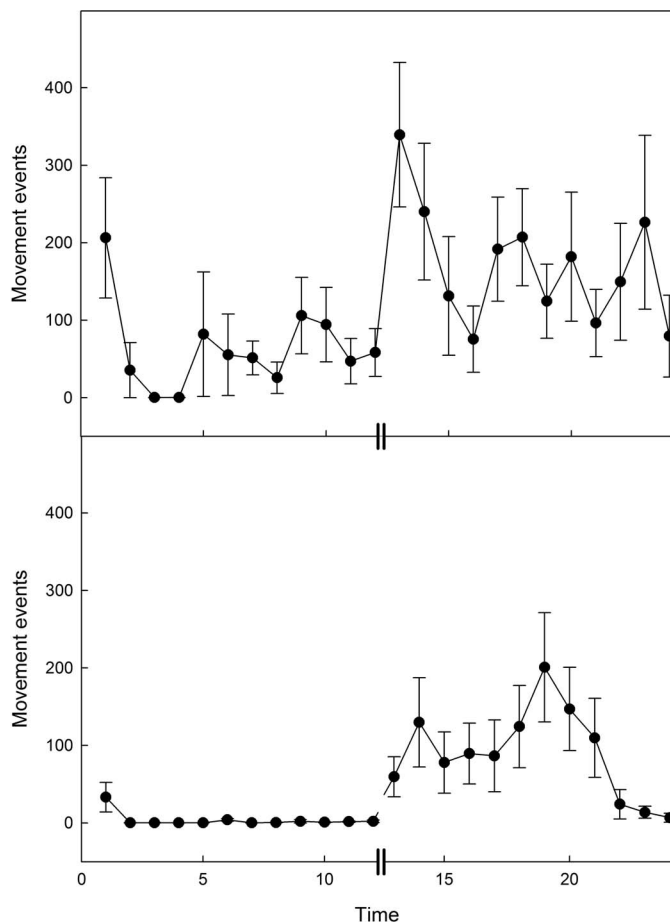


Figure 1.—Activity pattern of *Loxosceles laeta* (upper) and *Scytodes globula* (lower) measured as number of movement events (ME) during a L:D/12:12 cycle. X-axis is measured in hours; vertical bars indicate onset of the 12-hour dark period.

## DISCUSSION

Although there are previous reports pointing to *L. laeta* and *S. globula* as nocturnal species, there are no previous systematic studies of the chronobiology of these species. In this study, both species entrained to a circadian cycle light:dark (12:12), and their locomotor activity was mainly restricted to the dark phase. Also, no differences in activity between males and females were observed even when experiments were performed in the reproductive period. This contrasts with previous reports showing greater cursorial activity of males than females, which has been attributed to the active search for these during reproductive period (Gonzaga et al. 2007). However, differences were observed in the proportion of activity of the spider species during the photophase, with 4.1% and 26.4% for *S. globula* and *L. laeta*, respectively.

The negligible diurnal activity of *S. globula* agrees with that reported for other nocturnal spiders such as araneomorph species of the genus *Cupiennius* Simon, 1891, in which only 4.1% of the activity of males and 8.7% of female activity occurs during the day (Ortega-Escobar 2002). However, the low level of diurnal activity of *S. globula* contrasts with that almost six times greater exhibited by *L. laeta*. Activity during

daylight hours in predominantly nocturnal species has been previously described in spiders. For example, a study on *Tetragnatha montana* Simon, 1874, and *Dolomedes fimbriatus* (Clerck, 1757) based on field observations showed that both species can extend their activity in daylight hours, especially in times of food shortage (Horn 1969).

Even though both species studied here developed their main activity during dark period, they differed in the degree of nocturnality, with *S. globula* showing a strong nocturnal periodicity and with *L. laeta*, a statistically significant nocturnality accompanied by some degree of activity during the light hours. This is in agreement with the assertion of individuals of other species of spiders which are not purely nocturnal or diurnal (Suter & Benson 2014) and the reported behavior of individuals of the brown recluse spider (*L. reclusa* Gertsch & Mulaik, 1940) which were observed active during the day, specially in darker places of a home (Cramer 2015). Moreover, the difference might also be attributable to different foraging strategies of these species. The spitting spider *S. globula* is more sedentary and employs a “sit and wait” strategy (Suter & Stratton 2005, 2009) which has been also described in the brown recluse spiders (Cramer 2015). Nevertheless, the Chilean recluse spider is probably a species which roams actively looking for prey and not only feeding on those who fall into their irregular webs (Suter & Stratton 2005, 2009). Furthermore, spiders of the genus *Loxosceles* Heiniken & Lowe, 1832, capture invertebrates in their webs or during ambulation (Fischer et al. 2006) and also use the strategy of cannibalism and consumption of dead prey in their path (Sandidge 2003; Fischer et al. 2006; Cramer 2008; Vetter 2011; Souza-Silva & Ferreira 2014). However, scavenging seems to be an opportunistic behavior which occurs under unusual circumstances only (Cramer 2008).

Overall, the difference of diurnal activity between the synanthropic species studied here is consistent with the common finding of the Chilean recluse spider wandering around during daylight hours while *S. globula* remains motionless in its typical rest position (Suter & Stratton 2005, 2009) in human habitations during the same period.

Another difference observed between *L. laeta* and *S. globula* was the shape of the nocturnal activity curves. *Scytodes globula* was bimodal with peaks of activity 2 and 7 hours after the beginning of the scotophase. This bimodal activity pattern has been described in some crepuscular mygalomorph spiders (Cloudsley-Thompson 2013). However, the activity of *L. laeta* during the dark phase was unimodal, reaching a maximum at the very beginning of the scotophase and maintaining sustained activity throughout this phase. A similar pattern has been recorded in *L. reclusa* (Cramer 2015).

The Chilean recluse spider may have higher energy requirements than *S. globula* because of its greater mass and more active strategy of foraging in environments of low prey availability such as anthropogenic environments (Schochat et al. 2004; Van Nuland & Whitlow 2014). This fact together with a higher response threshold to light intensity might drive the extended activity to daylight. Species with some degree of diurnal activity are expected to have a higher threshold of light intensity than those having strictly nocturnal activity. Preliminary data show that this is the case for *L. laeta* and *S. globula*, with light intensity thresholds producing a decrease or



cessation of activity significantly at greater than 20 lux for the Chilean recluse spider and *S. globula* stopping its activity long before reaching that value (unpublished data). Whatever the reason, comparatively *L. laeta* shows physiological characteristics such as less sensitivity to the thermal environment and a higher rate of water loss which made this species more self-reliant than *S. globula* (Canals et al. 2015c).

Both species studied here show an extensive and remarkable thermal niche overlap (Alfaro et al. 2013) which implies that their micro-habitats are also similar (Canals & Solís 2013) and increases the probability of meeting and competitive interactions. Taken together, these facts and the results of the daily pattern of locomotor activity studied here, it is very possible that niche overlap encircle many other niche variables determining strong competitive interactions between Chilean recluse spider and *S. globula* spiders, especially in domestic environments. Nevertheless, the diurnal activity presented by individuals of *L. laeta* may decrease the probability of encounter with the strongly nocturnal araneophagic *S. globula*.

Spiders in our experimental arena faced an unusual environment, bare and without shelter, very different to those where these species are commonly found. Clearly, this fact preclude direct extrapolation to human-structured environment where some metrics like movement events may be lower, because availability of many places to hide. However, in spite of this, our results give strong evidence of real differences in locomotor activities between both species and the way this activity develops during light and dark periods.

Previous studies contributed information about the use of space (“where”) by these spiders that would help to increase the efficiency of sanitary control (Vetter & Rust 2008, 2010). Complementarily, the knowledge of the use of time by these synanthropic species helps to understand “when” it is most likely these species will interact and improve measures of control.

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