

## Microhabitat use in the amblypygid *Paraphrynus laevifrons*

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**Abstract.** Amblypygids (Order: Amblypygi) can be found across different habitat types, each with very different microhabitat structure, including rainforests, deserts, and caves in the tropics and subtropics. Most prior studies on amblypygid microhabitat use have focused on characteristics of trees and their relationship with amblypygid abundance, though many species regularly occupy refuges away from trees. Here we explore microhabitat use in the amblypygid *Paraphrynus laevifrons* Pocock, 1894 through mark-recapture surveys conducted along creeks and trails in a secondary forest in southeastern Costa Rica. We identified (1) microhabitat characteristics associated with abundance of *P. laevifrons* and (2) resighting ratio—the likelihood of finding individual *P. laevifrons* over multiple nights, potentially in association with a particular area (a putative territory). We measured four microhabitat characteristics: (i) number of visible refuges, (ii) surface area of vertical substrate, (iii) estimated plant cover of substrate, and (iv) presence/absence of an overhang. We found that the number of *P. laevifrons* sighted did not differ across wet and dry seasons, but *P. laevifrons* were sighted in greater numbers in creeks than trails. The abundance of *P. laevifrons* was positively affected by the presence of overhangs, there was no effect of plant cover, and the positive effect of number of refuges was stronger in trails, where overhangs were less common, than in creeks. Our results support earlier studies showing that amblypygids can be found more abundantly in areas with greater available refuges and potential shelter, suggesting that predation may be a strong source of selection on amblypygid microhabitat use.

**Keywords:** Microhabitat preference, natural history, whip spider, movement patterns

Arachnids occupy a tremendous range of habitats, and habitat and microhabitat use has been studied in diverse arachnid taxa. While broad-scale studies of spiders have demonstrated how species presence and density differ across habitat types (Jordan et al. 1994; Aiken & Coyle 2000; Bonte & Maelfait 2001; Pearce et al. 2004; Lapinski & Tschapka 2013), finer-scale studies show how factors such as prey abundance (Harwood et al. 2003; Johnson et al. 2011), anthropogenic building materials (Fischer et al. 2005), proximity to water (DeVito et al. 2004; Lapinski & Tschapka 2014), temperature, and humidity (Yáñez & Floater 2000) affect microhabitat preference within these areas. Further, predictable changes in the environment may lead to seasonal patterns of (micro)habitat use within species (Arango et al. 2000). Together, such studies provide important information regarding the ecology and natural history of spiders, yet much of this basic information is lacking in other arachnid groups.

Like their spider relatives, amblypygids (Order: Amblypygi) are found across a wide range of habitats, including rainforests, deserts, and caves in the tropics and subtropics (reviewed in Weygoldt 2000). Their dorsoventrally flattened bodies allow them to occupy narrow crevices in and around rocks, fallen logs, and at the base of trees (Weygoldt 1977, 2000). Their antenniform front legs are covered in mechano- and chemosensory hairs used in olfaction, touch, and contact chemoreception (Weygoldt 2000; Foelix & Hebets 2001; reviewed in Santer & Hebets 2011), and have been demonstrated to facilitate the learning and discrimination of tactile cues associated with available refuges (Santer & Hebets 2009).

Amblypygids are presumed to be territorial (Hebets 2002; Porto & Peixoto 2013; Chapin & Hill-Lindsay 2015). Previous studies in the amblypygid *Phrynus pseudoparvulus* Armas & Viquez, 2002, for example, have shown that individuals not only occupy home refuges for extended periods of time (weeks–months) but they may travel far distances away from

this home refuge, only to return weeks later (Hebets 2002). Further field studies in the amblypygid *Heterophrynus longicornis* Butler, 1873 suggest that individuals may select and defend territories based on the presence of burrows; released individuals were found and remained at sites with burrows present, and when large individuals were removed from a putative territory, smaller individuals took their place (Porto & Peixoto 2013). In addition to field data on movement patterns, ritualized agonistic interactions are commonly observed between conspecific amblypygids; and these ritualized displays during intrasexual interactions have been hypothesized to play a role in defense of a home territory (*Phrynus marginemaculatus* C.L. Koch, 1840, Fowler-Finn & Hebets 2006; *Heterophrynus longicornis*, Porto & Peixoto 2013; *Phrynus longipes* Pocock, 1894, Chapin & Hill-Lindsay 2015; reviewed in Santer & Hebets 2011 and Chapin & Hebets 2016). Given the suggestion of territoriality and the observations of agonistic interactions between conspecifics in some species, it seems likely that there is competition for resources in their environment.

Previous studies on amblypygid microhabitat use have found that amblypygid species in New World tropical and subtropical rainforests prefer trees that are large and/or have an abundance of burrows or refuges (Hebets 2002; Dias and Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013; Chapin 2014; Curtis & Bloch 2014). Hebets (2002) found a positive correlation between the number of *P. pseudoparvulus* sighted and the surface area of trees; individuals were more likely to be resighted on trees with greater surface area, moss cover, and buttressing. Carvalho et al. (2012) found *H. longicornis* more abundantly in forest fragments with a greater number of trees and on trees with termite nests; however, individuals were found more frequently on relatively small trees (DBH between 10–50 cm). Together, these studies suggest that multiple microhabitat characteristics may affect

habitat use in different ways for different amblypygid species. But while most prior studies have focused on the characteristics of trees within primary lowland tropical forests and their relationship with amblypygid abundance, many amblypygid species regularly occupy refuges away from trees (reviewed in Weygoldt 2000) and/or occupy multiple microhabitat types. In the present study, we performed mark-recapture surveys across two microhabitat types (creeks and trails) within a secondary lowland tropical rainforest in southeastern Costa Rica for a widespread amblypygid species, *Paraphrynus laevifrons* Pocock, 1894.

*Paraphrynus laevifrons* is found across Costa Rica and into Panama (Mullinex 1975; Viquez pers. comm.). Individuals can be found on vertical surfaces, including the base of trees and the sides of creeks, in tropical wet forests (Corey pers. obs.). Through our surveys, we aimed to identify (1) microhabitat characteristics associated with abundance of *P. laevifrons*, and (2) resighting ratio – i.e., the likelihood of finding individual *P. laevifrons* over multiple survey nights, potentially in association with a particular area (a putative/potential territory).

## METHODS

**Study area.**—This study took place at Las Cruces Biological Station in Coto Brus county, Costa Rica. The areas surrounding the biological station are predominantly made up of selectively-logged primary, tropical wet forest and secondary forest. To examine microhabitat use within and across creeks and trails, we established three non-overlapping survey plots, each containing a paired creek and trail transect. In total then, we surveyed three creek transects and three trail transects (3 pairs of transects; one pair within a “survey plot”). Two survey plots had transects that were perpendicular to each other, and one survey plot had transects that were parallel.

Creek and trail transects were 50 m x 3 m, which in practice included only one side of a given creek or trail due to their width. We included the side with more measurable vertical substrate, and therefore more potential microhabitat for *P. laevifrons*, following our preliminary observations that *P. laevifrons* are found on vertical surfaces. To obtain more accurate measurements of microhabitat characteristics and locations of individual *P. laevifrons*, we divided each individual creek and trail transect into approximately 5 m-long “survey grids,” with a total of ten survey grids nested within each transect. Creek and trail transects within a survey plot were close (as close as 1 m at some points), but did not directly overlap, leaving the possibility that individuals could move between transects. During the course of our surveys, and during additional fieldwork at the station, *P. laevifrons* was the only amblypygid species identified.

**Microhabitat characteristics.**—In creek and trail transects, we measured the following four microhabitat characteristics: (i) number of visible refuges, (ii) surface area of vertical substrate (i.e., area of exposed creek/trailside that individuals could climb on), (iii) estimated plant cover of substrate, and (iv) presence/absence of an overhang.

We define a (i) visible amblypygid refuge as an area large enough for at least a small juvenile *P. laevifrons* to enter and remain covered (e.g., an abandoned animal burrow or small crevice) with only one visible entrance in the side of the

vertical substrate. To estimate (ii) surface area of the vertical substrate (i.e., the amount of exposed and/or plant-covered soil on creek/trailside along transects), we measured the height of transects at two points within each survey grid – approximately 1 meter from the start and from the end of the survey grid – and averaged the two heights. We then multiplied the average height by the width of each survey grid (approximately 5 meters) to obtain vertical surface area. We only found *P. laevifrons* on vertical surfaces in preliminary surveys, and therefore only sampled these vertical surfaces in this study. Similar to Hebets (2002), we estimated (iii) plant cover within survey grids as the amount of living and dead plant material (leaves and stems) covering the vertical substrate, in the following ranges: 0–25%, 25–50%, 50–75%, and 75–100% cover. We considered (iv) an overhang to be present in an individual survey grid if the majority of vertical substrate (over 50% of the survey grid width) had above it a horizontal surface that protruded beyond the base of the creek/trailside, usually comprised of compacted soil and roots.

**Mark-recapture surveys.**—We performed mark-recapture surveys between 11–21 August 2015 (wet season), and 6–10 January 2016 (dry season). Surveys took place at night, between 2000 and 0500 hours (wet season) and 1925 and 2400 hours (dry season). We surveyed the same six transects (creek = 3, trail = 3) across wet and dry seasons. Across seasons, each creek and trail transect was surveyed three times over three different nights. During the wet season, we surveyed transects in similar microhabitats in a given night – i.e., all 3 creek transects or all 3 trail transects – for a total of three survey nights per transect (and per microhabitat type). During the dry season, we surveyed transects within a shared survey plot (creek + trail) on any given night and 2–3 of our survey plots were surveyed per night. This difference in surveying procedure was due to greater time constraints on fieldwork in the wet season and a reassessment of best survey protocols following the wet season surveys. Despite subtle differences in methodology, we have a total of 3 survey nights for each of our 3 creek and 3 trail transects for both wet and dry seasons.

We surveyed for *P. laevifrons* using a headlamp with white light, and examined each individual survey grid from beginning to end at least twice per survey, moving from the beginning to the end of the transect. The total time spent conducting nightly surveys varied depending on the number of *P. laevifrons* sighted and captured. Whenever we found an individual, we recorded the survey grid that it was located in as well as a general description of its location (e.g., under an overhang, adjacent to a small crevice, on an exposed root). We opportunistically hand-captured as many individuals as possible and recorded their cephalothorax width (in mm, using Control Company Traceable® 150 mm digital calipers), age class (juvenile or adult), and labeled them with an identification code using Sharpie non-toxic oil-paint markers. Age class was identified using both individual size and coloration. Juveniles of *P. laevifrons* have pronounced red pedipalps and a yellow perimeter around the dorsal side of their cephalothorax, while adults typically have pedipalps that are gray-brown, similar to the rest of their bodies, and a more subdued color to the perimeter of their cephalothorax (Corey pers. obs.). Previous studies show that related amblypygids reach sexual maturity when they have achieved a cephalotho-

Table 1.—Summary of mark-recapture surveys for juvenile and adult *Paraphrynus laevisfrons* along creek and trail transects during wet (August) and dry (January) seasons. See Methods for details on calculating ratio of individuals resighted (here expressed as a percentage), estimated population size and estimated population density (individuals/m<sup>2</sup>),  $\pm$  standard deviation. Where standard deviations are not shown, we could only calculate one estimate pool as no individuals were resighted in that sample (see Methods). Individuals were more often resighted in trails than creeks, but there were no differences across age classes and seasons (see Table 3).

Age	Season	Micro-habitat	Number Sighted	Number Captured	Percent Resighted	Estimated Pop. Size	Estimated Pop. Dens.
Juvenile	Wet	Creek	56	23	25.0%	170.0 $\pm$ 116.0	0.61 $\pm$ 0.42
		Trail	27	9	100.0%	17.3 $\pm$ 1.9	0.11 $\pm$ 0.02
		Total	83	32	55.0%	91.7 $\pm$ 111.0	0.21 $\pm$ 0.02
	Dry	Creek	55	25	35.0%	142.5 $\pm$ 111.0	0.51 $\pm$ 0.40
		Trail	12	5	20.0%	35.0	0.21
		Total	67	30	32.0%	249.9 $\pm$ 219.4	0.57 $\pm$ 0.06
Adult	Wet	Creek	44	22	27.3%	97.0 $\pm$ 18.4	0.35 $\pm$ 0.07
		Trail	12	7	80.0%	3.8 $\pm$ 1.8	0.02 $\pm$ 0.01
		Total	56	29	43.8%	91.8 $\pm$ 11.6	0.21 $\pm$ 0.03
	Dry	Creek	48	20	29.4%	76.5 $\pm$ 61.5	0.28 $\pm$ 0.29
		Trail	11	3	33.3%	7.0	0.04
		Total	59	23	30.0%	73.9 $\pm$ 26.3	0.17 $\pm$ 0.06

rax width greater than 10 mm (*Phrynus marginemaculatus*, Weygoldt 2002); therefore, we identified all captured individuals with a cephalothorax width greater than 10 mm to be adults, and those with smaller cephalothoraxes to be juveniles.

Resighting ratio was calculated for individual age classes and seasons by adding the number of previously marked individuals sighted on the second and third (final) survey nights across transects, and dividing by the number of individuals marked on the first and second survey nights. The estimated population size across transects was calculated using the Multiple Lincoln-Petersen estimator (following Grimm et al. 2014). The Multiple Lincoln-Petersen estimator pools multiple survey samples into  $k - 1$  estimates (where  $k$  is the number of survey samples) from which the average and variance are then calculated. In our study,  $k = 3$  samples per microhabitat per season, so we therefore have two estimates that we use in calculating the average population size and its variance (here expressed as standard deviation). In the Lincoln-Petersen model,  $N = (n_1 * n_2) / m_2$ , where  $N$  is the estimated population size,  $n_1$  is the number of individuals marked in an initial survey (the number of captured *P. laevisfrons* on the initial survey night in a transect),  $n_2$  is the size of the subsequent survey sample (the total number of sighted *P. laevisfrons* in the subsequent survey night in a transect), and  $m_2$  is the recapture ratio (the number of previously marked individuals resighted in a subsequent survey night in a transect). For the Multiple Lincoln-Petersen estimator, we formed two different pools, where (1) survey nights 1 and 2 are combined as the initial survey and survey night 3 is the subsequent survey, and (2) survey night 1 is the initial survey and survey nights 2 and 3 are combined as the subsequent survey. Population densities of *P. laevisfrons* (individuals/m<sup>2</sup>) across age classes (juvenile/adult), microhabitats, and seasons were estimated by dividing population sizes using the Multiple Lincoln-Petersen estimator by the surface area across transects (Creek = 277.5 m<sup>2</sup>, Trail = 163.5 m<sup>2</sup>, Total = 441 m<sup>2</sup>).

The Lincoln-Petersen model assumes that (i) the population is closed to recruitment, death, immigration, and emigration, (ii) capture probability is equal among animals in each sample, and (iii) all marked animals are found in subsequent surveys

(reviewed in Pine et al. 2003). This estimator allows for the pooling of mark-recapture data across greater than two survey samples, and is therefore more robust to violations of the assumption that capture probability is equal among animals in each sample (assumption ii above), by increasing the capture probabilities and decreasing the range of capture probabilities (Grimm et al. 2014). Nevertheless, given that these are strict assumptions, and likely to be violated, we present these data as rough, preliminary estimates.

**Statistical analyses.**—Differences in abundance across seasons (wet vs. dry), and differences in the number of refuges, density of refuges, and plant cover across microhabitat types, were compared using a Mann-Whitney U Test. We used a chi-squared test to compare differences in the frequency of overhang presence across microhabitat types.

We analyzed the effects of microhabitat characteristics on abundance of *P. laevisfrons* using a negative binomial-distributed, generalized linear mixed model. The response variable in the model was the number of individual *P. laevisfrons* (both adults and juveniles) sighted per survey grid on a given survey night. We use this measure of abundance, individuals sighted *per survey grid*, because this is the spatial scale at which we measured the microhabitat characteristics. The model contained fixed effects terms for the number of refuges, vertical surface area, estimated plant cover (as a continuous variable, using the midpoint of each estimated range), microhabitat type (creek/trail), and overhang (present/absent). We specified an interaction term between microhabitat type and overhang presence, and microhabitat type and number of refuges following preliminary analyses. Given that vertical surface area differed across microhabitat types, we use surface area as a means of controlling for differences in sampling effort across microhabitat types. That is to say, we can examine how much variation in the abundance of *P. laevisfrons* can be attributed to variation in microhabitat characteristics after controlling for the variation in response to surface area. The model also contained a random effects term for survey night nested within transect, specifying randomly varying intercepts, to control for repeated sampling of the same transects across survey nights (Table 2).

Table 2.—Effects of microhabitat characteristics on abundance of *Paraphrynus laeivifrons* (negative Binomial-distributed, generalized linear mixed model). Estimates are the number of individuals found per 5 meter long survey grid along study transects. Values in **bold** are significant at  $p = 0.05$ .

Fixed Effects	Estimate	Std. Error	z value	$P(> z )$
Overhang (Present)	1.138	0.245	4.64	<b>&lt;&lt;0.001</b>
Microhabitat (Trail)	-0.847	0.351	-2.41	<b>0.016</b>
Number of Refuges	0.023	0.012	1.91	0.056
Vertical Surface Area	0.049	0.018	2.65	<b>0.008</b>
Plant Cover	-0.002	0.003	-0.54	0.593
Microhabitat * Overhang	-0.349	0.472	-0.74	0.460
Microhabitat * Number of Refuges	0.038	0.018	2.11	<b>0.035</b>
Random Effects	Variance	Std. Dev.		
Survey Night : Transect	<<0.001	<<0.001		
Transect	0.008	0.089		

We used a binomial-distributed generalized linear mixed model with a response variable of resighting a marked individual (yes/no) to examine differences in the ratio of resighted individuals. This model contained fixed effects terms for the age class of individuals (juvenile/adult), season (wet/dry), and microhabitat type (creek/trail). We also included a random effects term for transect surveyed, specifying randomly varying intercepts (Table 3). All statistical analyses were performed using R version 3.3.2.

## RESULTS

The number of *P. laeivifrons* sighted did not significantly differ across seasons (Mann Whitney U test,  $W = 16,449$ ,  $P = 0.770$ ; Fig. 1), but *P. laeivifrons* were sighted in greater numbers in creeks than trails (Tables 1, 2). During the wet season, 139 *P. laeivifrons* (57 adults and 82 juveniles) were sighted, of which 61 were captured and labeled. During the dry season, 126 *P. laeivifrons* (59 adults and 67 juveniles) were sighted, of which 51 were captured and labeled (Table 1).

The number of visible refuges was greater in creeks than trails (Mann Whitney U Test,  $W = 657$ ,  $P = 0.002$ ). However, refuge density did not differ between creeks and trails (Mann Whitney U Test,  $W = 455$ ,  $P = 0.5996$ ), suggesting that this pattern is driven by differences in vertical surface area, which was greater in creeks. Plant cover did not differ between creeks

Table 3.—Differences in resighting ratio as affected by age class (juvenile/adult), microhabitat type (creek/trail), and season (wet/dry) (Binomial-distributed, generalized linear mixed model). Estimates are probability of resighting a previously marked individual *Paraphrynus laeivifrons*. Values in **bold** are significant at  $p = 0.05$ .

Fixed Effects	Estimate	Std. Error	z value	$P(> z )$
Microhabitat (Trail)	1.417	0.553	2.58	<b>0.010</b>
Age (Juvenile)	0.186	0.491	0.38	0.705
Season (Wet)	0.592	0.491	1.21	0.228
Random Effects	Variance	Std. Dev.		
Transect	0	0		

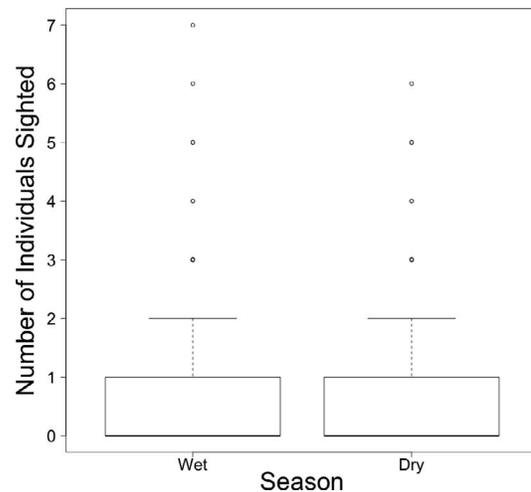


Figure 1.—Box-and-whisker plot of individual *Paraphrynus laeivifrons* sighted in a given 5 meter-long survey grid per transect survey. The average number of *P. laeivifrons* sighted did not differ between the wet season (August) and the dry season (January). Dots represent survey grid observations in which the number of individuals sighted was greater than the 75<sup>th</sup> percentile for a given season.

and trails (Mann Whitney U Test,  $W = 384.5$ ,  $P = 0.3139$ ) and overhangs were present more frequently in creeks than trails (Chi-squared test,  $\chi^2 = 12.129$ ,  $P = 0.0005$ ).

The differences in surface area between microhabitat types explained a large amount of the variation in the abundance of *P. laeivifrons* (Table 2). After controlling for this difference, we found that the total abundance of *P. laeivifrons* was positively affected by the presence of an overhang (Fig. 2, Table 2), while there was no effect of plant cover on abundance (Table 2). The number of refuges alone was not found to be a significant predictor of *P. laeivifrons* abundance in our analyses ( $P = 0.056$ ), though we did find a significant interaction between microhabitat type and the number of refuges (Table 2). Specifically, we found that the positive effect of refuge number on abundance of *P. laeivifrons* was stronger in trails than in creeks.

Resighting ratio was lower in creeks than trails, but did not differ across age classes or seasons (Tables 1, 3). All individuals resighted in the same season were found in the same or a neighboring survey grid. We identified two adult *P. laeivifrons* that were observed in both seasons – one of these individuals was found in the same transect, and the other was found in the creek transect neighboring the trail transect in which it was originally sighted.

Our preliminary findings on population density, as estimated using the Multiple Lincoln-Petersen estimator, suggest that density may differ across age classes and microhabitat types (Table 1), though these patterns may be similar across wet and dry seasons. Specifically, juvenile *P. laeivifrons* may be found in greater population densities than adults, and both age classes are found in greater densities in creeks than trails.

Given that we opportunistically collected individuals and measured their size, we were able to obtain additional information regarding the phenology of *P. laeivifrons*. We found one adult carrying brood on its opisthosoma during our January surveys and we captured 5 juveniles with cephalo-

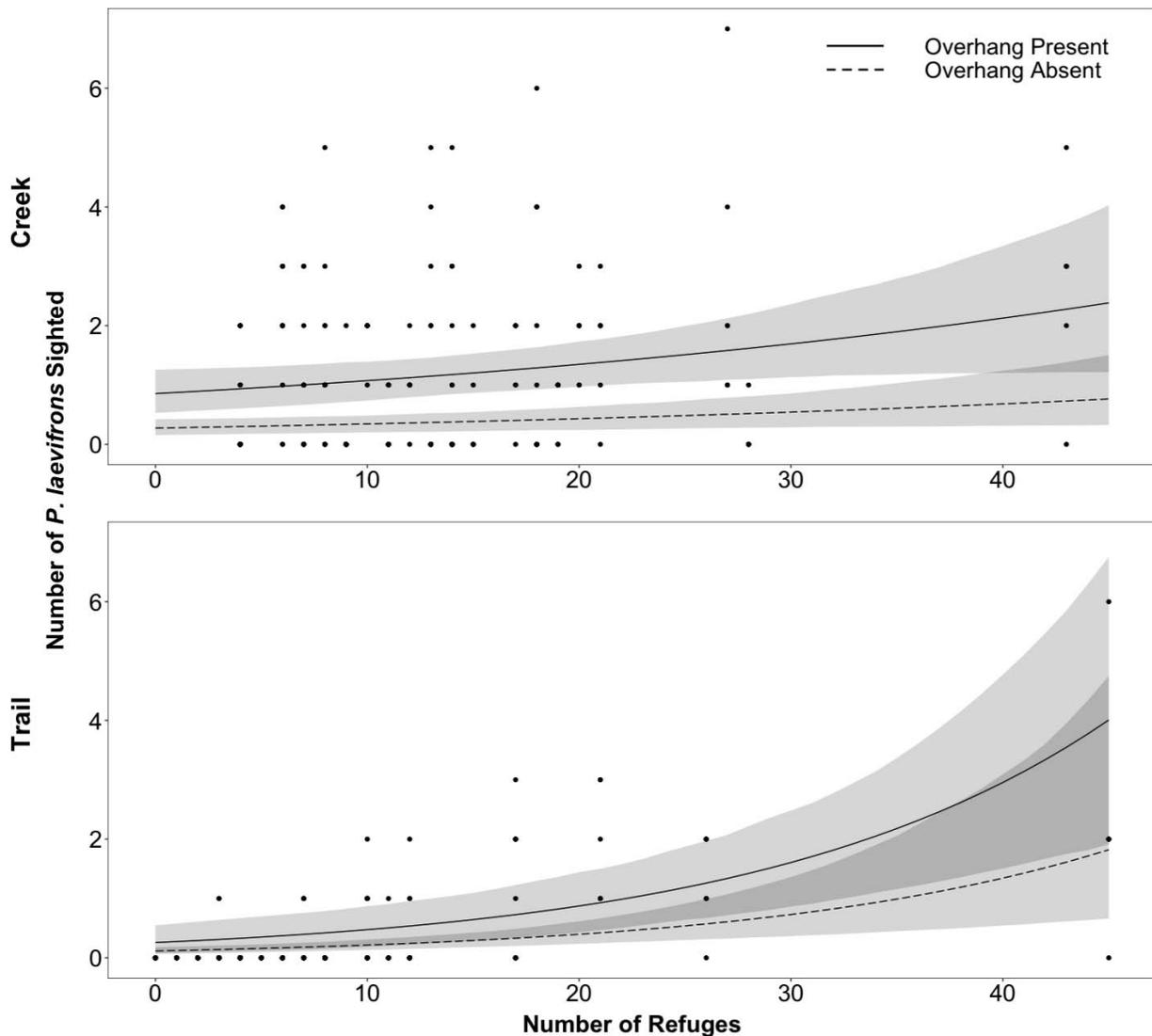


Figure 2.—The number of *P. laevifrons* sighted during surveys within a five meter-long survey grid was positively affected by the number of visible refuges and the presence of an overhang over the majority of the survey grid. There was an interaction between the number of refuges and microhabitat type, but no interaction between overhang presence and microhabitat type. Each dot represents an individual survey night observation for a grid containing the number of refuges indicated by the x – axis. Each individual survey grid was examined three times each in the wet and dry seasons, all on separate nights. Shaded areas represent 95% confidence intervals ( $n = 1000$  bootstrap simulations per prediction line).

thorax widths between 3 and 4 mm in January (minimum = 3.41 mm), while no individuals this small were found during our surveys in August (minimum = 4.76 mm) (Fig. 3). These are likely very young individuals, who may have recently left their mother's opisthosoma (Weygoldt 2000). We also found multiple small juveniles in the same survey grid as a single, large adult several times during January surveys. In subsequent collecting trips (3–11 June 2016), two females with egg sacs were collected, and three individuals formed egg sacs while in captivity between June and mid-August.

In addition, we collected three individuals during the wet season that were carrying the pupae of parasitic flies (family: Chloropidae) on the dorsal side of their opisthosoma (including one individual with its dorsal prosoma covered in pupae as well). We housed individuals until the adults eclosed,

and identified these flies as *Pseudogaurax sp.* based on previous descriptions by Viquez & DeArmas (2009).

## DISCUSSION

Individuals of the amblypygid *Paraphrynus laevifrons* were commonly found along creeks and trailsides at Las Cruces Biological Station in a tropical rainforest in Costa Rica. Our surveys indicated that individuals were in higher abundance along creek transects as compared to trail transects (Table 2), and this difference was consistent across wet and dry seasons. Our observed differences in abundance are likely driven, in part, by differences in microhabitat characteristics – specifically surface area, overhangs, and refuges. Differences in surface area between creeks and trails explained a large

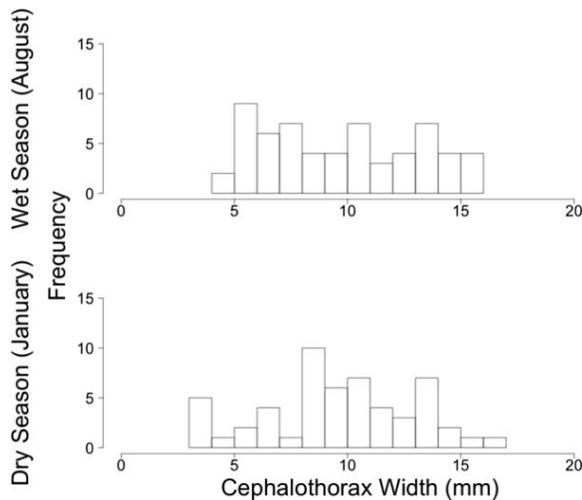


Figure 3.—Cephalothorax widths (mm) of *Paraphrynus laevifrons* captured, marked, and released during mark-recapture surveys in the wet season (August) and dry season (January). The range of sizes was greater in the dry season.

amount of the variation in the abundance of *P. laevifrons* (Table 2). After controlling for this confounding variable, we found that the presence of overhangs was the best predictor of amblypygid abundance, with a greater frequency of overhangs associated with more amblypygids. While the number of refuges alone was not predictive of amblypygid abundance, we found a significant interaction term between microhabitat type and number of refuges (Table 2). Specifically, the positive effect of refuges on abundance was greater in trails than in creeks. While the total number of refuges was greater in creeks, refuge density (the number of refuges divided by vertical surface area) did not differ across microhabitat types, suggesting that the greater difference of vertical surface area in creeks may be driving this difference. Given that overhangs are more common in creeks than trails (60% of creek survey grids had overhangs, compared to only 13.3% of trail survey grids), but refuges are similarly dense in creeks and trails, refuges may be a more important source of shelter for *P. laevifrons* along trailsides than in creeks. That is, in the absence of potential cover provided by overhangs, refuges may play a stronger role in microhabitat selection for *P. laevifrons*. This would be consistent with previous studies that have demonstrated how the abundance of refuges plays an important role in microhabitat use in amblypygids (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013).

Our population density calculations suggest higher densities of juveniles than adults, with both age classes found in greater densities in creeks than trails (Table 1). The ratios of marked, resighted individuals were less in creeks than in trails, but did not differ across age classes or seasons (Tables 1, 3). The greater resighting ratio along trails may suggest greater site fidelity in this microhabitat (which has been demonstrated in other amblypygid species, see Hebets 2002; Hebets et al. 2014). Given that population densities of *P. laevifrons* appear to be lower along trails (see below), individuals may be able to maintain territories longer due to a reduced encounter rate with conspecifics. This hypothesis requires testing. Additionally, it is important to note that this study reflects a very small

time window of observation; further longer-term observations are required to generate a complete picture of microhabitat use and movement patterns in *P. laevifrons*.

Previous microhabitat preference studies on amblypygids in the genus *Heterophrynus* found that individuals prefer trees with buttressing and many crevices (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013; Chapin 2014). Our surveys focused solely on creeks and trails, as preliminary forest transect surveys found only two individuals across 71 trees (unpublished data). The secondary forest has been selectively logged at our field site, and presumably the largest trees (containing potentially high-quality microhabitat) were removed. Selective logging has been demonstrated to decrease population density in the amblypygid *Phrynus longipes* (Bloch & Weiss 2002). Nonetheless, similar to other studies, our results suggest that diurnal refuges and microhabitat characteristics that offer shelter are the most important predictors of amblypygid abundance. At our field site, these characteristics are most prevalent along creeks. Future studies should examine microhabitat use in *P. laevifrons* in undisturbed primary forests, as our observations of microhabitat use may reflect the species' strategy for coping with anthropogenic disturbance rather than preferred microhabitat use.

Our present study focused only on the structural microhabitat characteristics that affect individual abundance in *P. laevifrons*. However, the physiology of *P. laevifrons* may interact with microhabitat characteristics to affect individual abundance and/or density. For example, Lapinski & Tschapka (2014) found that desiccation tolerance reflects the microhabitat association patterns of an assemblage of tropical wandering spiders – specifically, that semi-aquatic and forest-ground-dwelling spiders had high water loss rates and desiccation susceptibility, which is consistent with their humid microclimate. Such a limitation may well exist in *P. laevifrons*, as individuals are very sensitive to humidity while in captivity. While we did not measure how humidity differs across creeks and trails, or in areas within these microhabitats that vary in the number of overhangs or refuges, we suspect that humidity is higher in creeks than trails. Future work may confirm if desiccation tolerance and relative humidity may contribute to the abundance patterns we have observed.

Differences in abundance across microhabitat types are also likely influenced by factors such as prey and predator abundance (see Harwood et al. 2003). We have little beyond anecdotal evidence of the natural prey and predators of amblypygids, so collecting this information would be broadly applicable to amblypygid ecology (reviewed in Chapin & Hebets 2016). During our nightly surveys, we observed two *P. laevifrons* foraging – one capturing an ant (unknown species) on a trail transect and one consuming a cricket (unknown family) on a creek transect. We also observed numerous individuals consuming unidentifiable, macerated prey items. No instances of predation on *P. laevifrons* were observed over the course of this study.

Our opportunistic observations of individuals of different size throughout the seasons provide us some insight into potential reproductive seasons for *P. laevifrons*. First, during the dry season, we found multiple small juveniles in close proximity with a single, large adult. Previous studies on captive amblypygids suggest that there may be prolonged

mother-offspring-sibling associations (*Phrynus marginemaculatus* and *Damon diadema* Simon, 1876, Rayor & Taylor 2006) and offspring may be capable of kin recognition (*D. diadema*, Walsh & Rayor 2008). We propose that these small individuals were offspring of the adult female. Additionally, in subsequent collecting trips (3–11 June 2016), two females with egg sacs were collected, and three individuals formed egg sacs while in captivity between June and mid-August. While it is unknown how long eggs of *P. laevifrons* take to hatch, in other species, offspring emerge three to three and a half months after eggs are laid (Weygoldt 2000). Taken together, our observations suggest that *P. laevifrons* has a similar breeding season to that suggested for *Phrynus pseudoparvulus* – namely a primary breeding season from October to January, with some individuals breeding throughout the year (Hebets 2002).

In summary, this study adds to a slowly growing body of literature focused on amblypygid behavioral ecology (Chapin & Hebets 2016). Our results are consistent with earlier studies showing that amblypygids can be found more abundantly in areas with greater available refuges and potential shelter (Dias & Machado 2006; Carvalho et al. 2012; Porto & Peixoto 2013), suggesting that predation may be a strong source of selection on amblypygid behavior. Unfortunately, little is known about the nature or abundance of amblypygid predators. Future fieldwork exploring natural predator-prey interactions—focusing on amblypygids as both predators and prey—as well as territorial behavior will help place our microhabitat use data in the broader context of amblypygid natural history.

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#### LITERATURE CITED

- Aiken, M. & F.A. Coyle. 2000. Habitat distribution, life history and behavior of *Tetragnatha* spider species in the Great Smoky Mountains National Park. *Journal of Arachnology* 28:97–106.
- Arango, A.M., V. Rico-Gray & V. Parra-Tabla. 2000. Population structure, seasonality, and habitat use by the green lynx spider *Peucetia viridans* (Oxyopidae) inhabiting *Cnidioscolus aconitifolius* (Euphorbiaceae). *Journal of Arachnology* 28:185–194.
- Bloch, C.P. & L. Weiss. 2002. Distribution and abundance of the whipspider *Phrynus* (Arachnida: Amblypygi) in the Luquillo Experimental Forest, Puerto Rico: response to natural and anthropogenic disturbance. *Caribbean Journal of Science* 38:260–262.
- Bonte, D. & J.P. Maelfait. 2001. Life history, habitat use and dispersal of a dune wolf spider (*Pardosa monticola* (Clerck, 1757) Lycosidae, Araneae) in the Flemish coastal dunes (Belgium). *Belgian Journal of Zoology* 131:145–157.
- Carvalho, L.S., J.O. Gomes, S. Neckel-Oliveira & N.F. Lo-Man-Hung. 2012. Microhabitat use and intraspecific association in the whip spider *Heterophrynus longicornis* (Arachnida: Amblypygi) in forest fragments formed by the Tucuruí Dam lake, Pará, Brazil. *Journal of Natural History* 46:1263–1272.
- Chapin, K.J. 2014. Microhabitat and spatial complexity predict group size of the whip spider *Heterophrynus batesii* (Amblypygi: Phrynidae) in Amazonian Ecuador. *Journal of Tropical Ecology* 30:173–177.
- Chapin, K.J. & E.A. Hebets. 2016. The behavioral ecology of amblypygids. *Journal of Arachnology* 44:1–14.
- Chapin, K.J. & S. Hill-Lindsay. 2015. Territoriality evidenced by asymmetric intruder-holder motivation in an amblypygid. *Behavioural Processes* 122:110–115.
- Curtis, C.A. & C.P. Bloch. 2014. Seasonal patterns of microhabitat selection by a sub-tropical whip spider, *Phrynus longipes* in the Luquillo Experimental Forest, Puerto Rico. *Journal of Arachnology* 42:126–129.
- DeVito, J., J.M. Meik, M.M. Gerson & D.R. Formanowicz. 2004. Physiological tolerances of three sympatric riparian wolf spiders (Araneae: Lycosidae) correspond with microhabitat distributions. *Canadian Journal of Zoology* 82:1119–1125.
- Dias, S.C. & G. Machado. 2006. Microhabitat use by the whip spider *Heterophrynus longicornis* (Amblypygi, Phrynidae) in Central Amazon. *Journal of Arachnology* 34:540–544.
- Fischer, M.L., S. Diniz & J. Vasconcelos-Neto. 2014. Do agonistic interactions underlie the segregation and relative abundances between two *Loxosceles* species (Araneae: Sicariidae)? *Journal of Medical Entomology* 51:547–559.
- Foelix, R. & E.A. Hebets. 2001. Sensory biology of whip spiders (Arachnida, Amblypygi). *Andrias* 15:129–140.
- Fowler-Finn, K.D. & E.A. Hebets. 2006. An examination of agonistic interactions in the whip spider *Phrynus marginemaculatus* (Arachnida, Amblypygi). *Journal of Arachnology* 34:62–76.
- Grimm, A., B. Gruber & K. Henle. 2014. Reliability of different mark-recapture methods for population size estimation tested against reference population sizes constructed from field data. *PLoS ONE* 9(6):e98840.
- Harwood J.D., K.D. Sunderland & W.O.C. Symondson. 2003. Web-location by linyphiid spiders: prey-specific aggregation and foraging strategies. *Journal of Animal Ecology* 72:745–756.
- Hebets, E.A. 2002. Relating the unique sensory system of amblypygids to the ecology and behavior of *Phrynus parvulus* from Costa Rica (Arachnida, Amblypygi). *Canadian Journal of Zoology* 80:286–295.
- Hebets E.A., E.J. Gering, V.P. Bingman & D.D. Wiegmann. 2014. Nocturnal homing in the tropical amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). *Animal Cognition* 17:1013–1018.
- Johnson, A., O. Revis & J.C. Johnson. 2011. Chemical prey cues influence the urban microhabitat preferences of Western black widow spiders, *Latrodectus hesperus*. *Journal of Arachnology* 39:449–453.
- Jordan, F., H.L. Jelks & W.M. Kitchens. 1994. Habitat use by the fishing spider *Dolomedes triton* in a northern Everglades wetland. *Wetlands* 14:239–242.
- Lapinski, W. & M. Tschapka. 2013. Habitat use in an assemblage of Central American wandering spiders. *Journal of Arachnology* 41:151–159.
- Lapinski, W. & M. Tschapka. 2014. Desiccation resistance reflects

- patterns of microhabitat choice in a Central American assemblage of wandering spiders. *Journal of Experimental Biology* 217:2789–2795.
- Mullinex, C.L. 1975. *Paraphrynus laevifrons*. Pp. 20–23. In Revision of *Paraphrynus* Moreno (Amblypygida: Phrynidae) for North America and the Antilles. Occasional Papers of the California Academy of Sciences, San Francisco.
- Pearce, J.L., L.A. Venier, G. Eccles, J. Pedlar & D. McKenney. 2004. Influence of habitat and microhabitat on epigeal spider (Araneae) assemblages in four stand types. *Biodiversity and Conservation* 13:1305–1334.
- Pine, W.E., K.H. Pollock, J.E. Hightower, T.J. Kwak & J.A. Rice. 2003. A review of tagging methods for estimating fish population size and components of mortality. *Fisheries* 28:10–23.
- Porto, T.J. & P.E.C. Peixoto. 2013. Experimental evidence of habitat selection and territoriality in the Amazonian whip spider *Heterophrynus longicornis* (Arachnida, Amblypygi). *Journal of Ethology* 31:299–304.
- Rayor, L.S. & L.A. Taylor. 2006. Social behavior in amblypygids, and a reassessment of arachnid social patterns. *Journal of Arachnology* 34:399–421.
- Santer, R.D. & E.A. Hebets. 2009. Prey capture by the whip spider *Phrynus marginemaculatus* C.L. Koch. *Journal of Arachnology* 37:109–112.
- Santer, R.D. & E.A. Hebets. 2011. The sensory and behavioural biology of whip spiders (Arachnida, Amblypygi). Pp. 1–64. In *Advances in Insect Physiology 41: Spider Physiology and Behaviour*. (S.J. Simpson, J. Casas, eds.). Elsevier, London.
- Viquez, C. & L.F. De Armas. 2009. Parasitismo en huevos de ambliopígidos (Arachnida: Amblypygi) por moscas Chloropidae (Insecta: Diptera). *Boletín Sociedad Entomológica Aragonesa* 45:541–542.
- Walsh, R.E. & L.S. Rayor. 2008. Kin discrimination in the amblypygid, *Damon diadema*. *Journal of Arachnology* 36:336–343.
- Weygoldt, P. 1977. Coexistence of two species of whip spiders (genus *Heterophrynus*) in the Neotropical rain forest (Arachnida, Amblypygi). *Oecologia* 27:363–370.
- Weygoldt, P. 2000. Whip Spiders (Chelicerata: Amblypygi): Their Biology, Morphology and Systematics. Apollo Books. Stenstrup, Denmark.
- Yáñez, M. & G. Floater. 2000. Spatial distribution and habitat preference of the endangered tarantula, *Brachypelma klaasi* (Araneae: Theraphosidae) in Mexico. *Biodiversity and Conservation* 9:795–810.

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