Convocatoria de ayuda a proyectos de investigación liderados por jóvenes investigadores (8ª ed., 2018)

1. Datos de identificación.

Título de la propuesta	Ant navigation in the Amazon forest: Which visual parts of the natural environment are used by *Gigantiops destructor* (Hymenoptera: Formicidae)?
Categoría	Ganando independencia
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2. Memoria Técnica. Actividades y resultados de investigación

2.1. Introducción (Planteamiento, objetivos y justificación)

Desert ants are the major model in navigation studies. However, desert environments are poor in horizontal visual cues, generally providing celestial information that help during navigation. But, what about ants living in visually rich environments? What they can see is the silhouette of the canopy, the geometric features around them and panoramic images of the horizon. Few studies have investigated how ants that inhabit visually noisy environments, such as forests, navigate, especially when considering tropical areas (Hölldobler, 1980; Narendra *et al.*, 2008). Horizontal and canopy cues have been demonstrated to be important for navigation of ants nesting in trees (*Paraponera clavata* and *Odontomachus hastatus*), but for ants nesting on the ground, there is a gap of knowledge regarding their orientation mechanisms (Ehmer, 1999; Rodrigues & Oliveira, 2014). *Gigantiops destructor* (Formicinae) forage within a complex environment: The Amazon forest. This species has characteristics that makes it an interesting research model in visual orientation. They inhabit partially sunny areas within the forest and are solitary diurnal foragers. In addition, they present the largest eyes of any ant species and do not use pheromones marks on their trails. So, what information does *Gigantiops destructor* extract from the visual scene when foraging or on the way back home?

The aim of this study was to begin to understand how ants use natural scenes for navigation inside complex environments, by testing the ability of *Gigantiops destructor* foragers to orient towards their nest (their most important place) when portions of the visual scene are obstructed.

For ants belonging to visually complex environments, there has been little research asking how they extract and use natural scenes for navigation. Behavioural studies of navigation help us to understand the sensory ecology of animals and how they deal with daily tasks. Analysing ants' perspective views of a habitat within which they navigate is crucial for interpreting behavioural mechanisms and adaptation. The navigation of *Gigantiops destructor* within their natural habitat provides an excellent model for studying visually guided orientation.

2.2. Descripción de la ejecución- Metodología

a. *Study area:*

The study was conducted from December 2017 to July 2018 at the Adolpho Ducke Forest Reserve (02°55' e 03°01' S, 59°53' e 59°59' W), which is part of the Long Term Ecological Research (LTER) Network (Fig. 1). The Reserve is managed by the National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil. During the study, I had the collaboration of Dr. Jorge Luiz P. de Souza and the master student Jefferson Telles da Silva, both from the INPA.

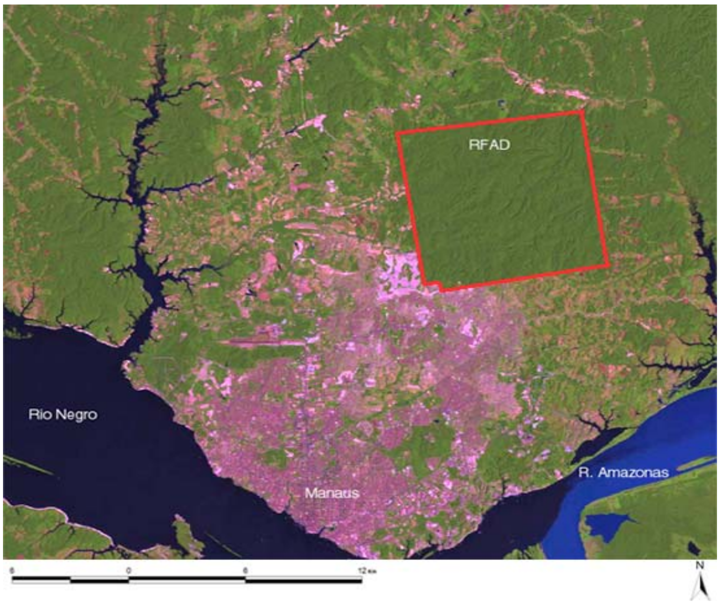


Figure 1: Location of the Ducke Reserve (RFAD)

b. *Sampling design:*

Prior to the beginning of experiments, we selected five nests of *Gigantiops destructor* with a minimum distance of 100 m from each other within the study area. Initially we conducted observations of the general behaviour by following individuals outside the nest. We determined their foraging routes, distances in relation to the nest (using a measuring tape), hours of activity and the collected food resources that individuals were bringing to nests. We decide to test individuals during experimental sessions on their pre-selected routes (instead of training them to forage in a selected route by us) in order to simplify the process. Thus, to be sure that individuals that were initially observed truly had established a concise foraging route, we followed individuals during five consecutive visits. As a visit, we considered the path Nest -> Final Foraging area -> Nest. By Final Foraging Area we mean the searching area, at the end of the path where, after having been travelled a certain distance from the nest, ants would start to systematically search for preys. We then selected 10 individuals per treatment (n = 50) and identified these individuals through non-toxic paint on their gaster using different colours. Ants were tested individually.

We followed the methodology described by Graham and Cheng (2009), who used a circular structure, called goniometer, consisting of an acrylic transparent disc of 60 cm in diameter, divided into 24 sections of 15° each (Fig. 2), with the nest always oriented at 0° (considered later during analyses as the sections between 24° and 1°). Besides the angular sections, the goniometer also presented three specific points: releasing point (from where ants were released), and 15 cm and 30 cm distances from the centre of the releasing area (Fig. 2), used to estimate ant's accuracy at different distances. Ants were individually released in the centre of the goniometer (± 5 cm of diameter). From this point, we recorded the time that ants took to cross the 15 cm and 30 cm lines, and the direction (angle) at each of those points. These data were collected through direct observation and through video recordings (Canon EOS 100D). Each individual was used during only one treatment and performed a total of 10 trials per treatment.

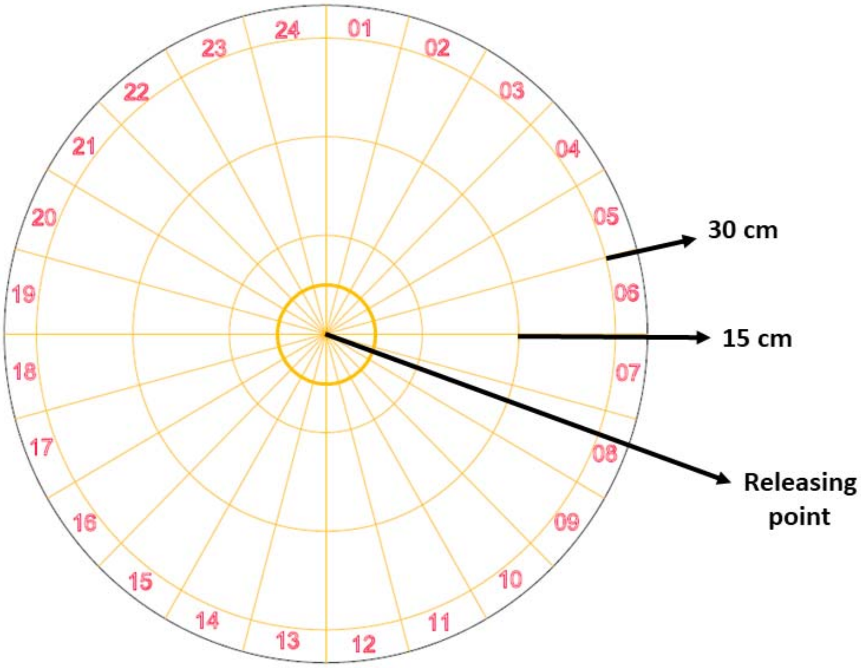


Figure 2: Schematic representation of the goniometer demonstrating the release point, 15 cm and 30 cm lines. Data of the direction and time spent by ants when crossing both lines were recorded.

c. General procedures:

After identifying the foraging routes and selecting the individuals, we offered a live termite as prey to ants, at their final foraging area. We repeated this procedure during five consecutive visits to confirm that ants accepted the prey and returned immediately to their nests following a relatively straight route, showing motivation. This phase consisted of training. After, we started the testing phase. In the testing phase, once the prey (resource from now on) was collected, we followed the individual until near the entrance of the nest, where it was captured using a small dark chamber. We applied this methodology to ensure that ants would not base their routes during experiments on the use of path integration, an information acquired by means of celestial compass and some form of step counting to estimate the distance travelled. These ants were considered “zero vector” ants, i.e., ants that have already completed their way back to the nest and have therefore redefined their path integration system to zero (vector is no long accumulated when the ant is back to its origin point). To prevent ants from using the observer as a point of reference, during each trip on the way back home, we ensured that our position relative to the arena was random. In addition, before each trip, the goniometer was cleaned with 70% alcohol to avoid the use of any chemical cue left during the previous visit (even though the literature states that *G. destructor* does not use pheromones as trail marks - Beugnon, Chagné, & Dejean, 2001). After capture, ants were released at an average distance of five meters from the nest entrance, considering their pre-established routes.

During the experimental sessions we applied the following treatments:

i. **Control**: to check whether ants can find their way back home assuming that they navigate using terrestrial cues. Ants were released in the centre of the goniometer in order to register their direction and time when returning to the nest;
ii. **Canopy blocked**: a black sheet (1cm^2 TNT fabric) positioned 60 cm above the goniometer, was used to block the canopy information upwards the goniometer area in order to test the behaviour of ants when visual cues from the canopy were blocked;
iii. **20 cm wall**: a 20 cm wall was used to block the panoramic view around the goniometer to understand whether ants could rely on celestial or canopy information in the absence of horizontal visual information;
iv. **10 cm gap + 10 cm wall**: to understand whether only the lower part of the visual panorama was sufficient to guide ants correctly toward the nest, we blocked the upper portion of the visual panorama with a wall of 10 cm;
v. **20 cm wall + Canopy blocked**: to understand how individuals behaved in the absence of panoramic and canopy visual information, both visual information were blocked.

d. Statistical analyses

To determine if foraging distances varied among nests, we performed a linear analysis considering the total distance travelled by the individuals (response variable) and the nest to which these individuals belonged (predictor variable). To test if the time ants took to cross the 30 cm line varied depending on the trip (first and last) and the treatment, we adjusted the time response variable to a linear mixed model (LMM, Bates & Maechler, 2013), using treatment and trip as predictors. In this model we used as a random factor the individual nested within the nest. Both foraging distance and time were logarithmically transformed to increase the linearity of models.

The orientation (angle direction) of ants was analysed through circular statistics. For this, the sections were transformed into angles, assuming 12 values (from 0° to 330°), with intervals of 30° between them. For example, sections 24 and 1 corresponded to the angle of 0° (nest direction), sections 2 and 3 to the angle of 30°, sections 4 and 5 correspond to the angle of 60°, and so on, up to the final angle of 330°. For each treatment, we used the Rayleigh test to determine if the directions followed by ants were evenly distributed (p> 0.05) or presented directionality. For the Rayleigh test, the length (*r*) and mean direction (μ) of the mean vector were considered. The length (*r*) can range from 0 to 1, where high values of *r* indicate that the observations are more clustered around the sample mean compared to small values (close to 0). If the data did not show uniformity (Rayleigh test), we tested for the existence of a significant clustering towards the nest direction (0°) using the V-test, with alpha established at p> 0.05. The V-test is a variant of the Rayleigh test, but instead of testing the null hypothesis of uniformity against a non-specific alternative, the V-test tests against an alternative hypothesis that the distribution is not uniform and has a specific mean, in our case, the direction of the nest (0 °).

Linear analyses were performed in the R software (R Core Team, 2015), using the *lme4* package (Bates & Maechler, 2013), as well as post-hoc analyses whenever there was a significant difference between groups. Post-hoc Tukey analyses were performed using the *multcomp* package (Hothorn, Bretz, & Westfall, 2008). Circular analyses were performed using the software Oriana, version 4.01 (Kovach Computing ServicesTM).

2.3. Resultados obtenidos (cumplimiento de objetivos)

During initial field observations of the behaviour of *Gigantiops destructor*, we saw that individuals established particular (idiosyncratic) foraging routes. These routes took them to specific points at different distances from the nest, ranging from 4.5 m to 21.7 m (n = 50), but there were no significant differences in mean distances (mean ± SD: 12.22 ± 3.6 m) between nests (F = 0.50, df = 4, p = 0.73). Throughout the observation period, individuals maintained a unique route, which had as ending point a place of active foraging and patrol, where the search for food was concentrated. Individuals were more active during the morning hours, with peak activity between 9:00 h and 11:00 h. At 17:00 h, there was practically no foraging activity (Fig. 2).

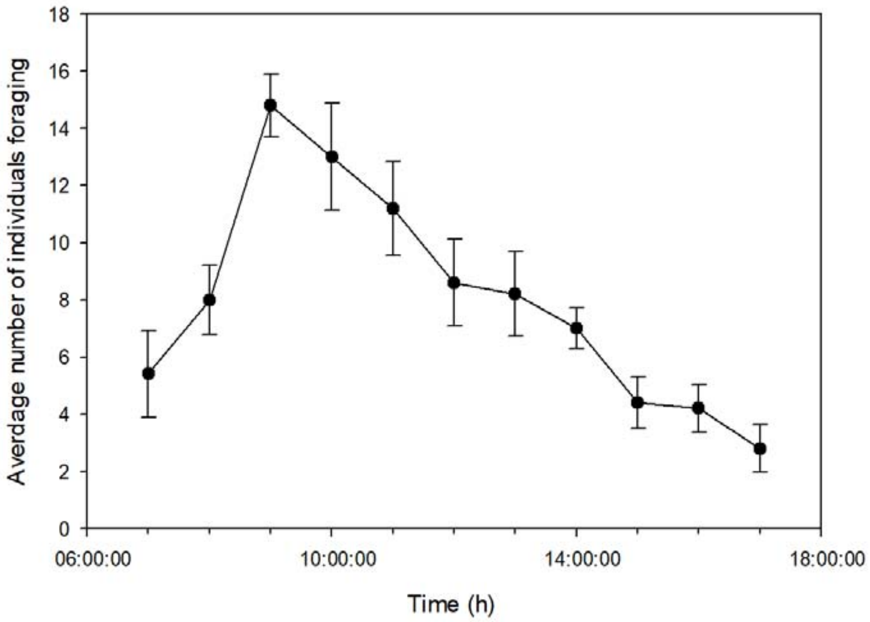


Figure 2: Mean and standard deviation of the foraging frequency of individuals per day, depending on the time. Observations were made for a period of five days, considering individuals from five nests.

Among the food resources transported to the nest we observe mainly invertebrates such as termites, spiders, beetles and even ants of other species. We did not record the transportation of any type of vegetal. In the analysis of the time spent by ants to cross the 30 cm line, during the first and last trips, we found that both the trip (F = 206, df = 1, p <0.01) and treatment (F = 25.28, df = 4, p <0.01) had a significant effect on time. Ants were faster during the last trip in comparison with the first in all treatments but performed better in the control, canopy blocked (CB) and in the 10 cm gap – wall situation (10 cm G-W) (Fig. 3).

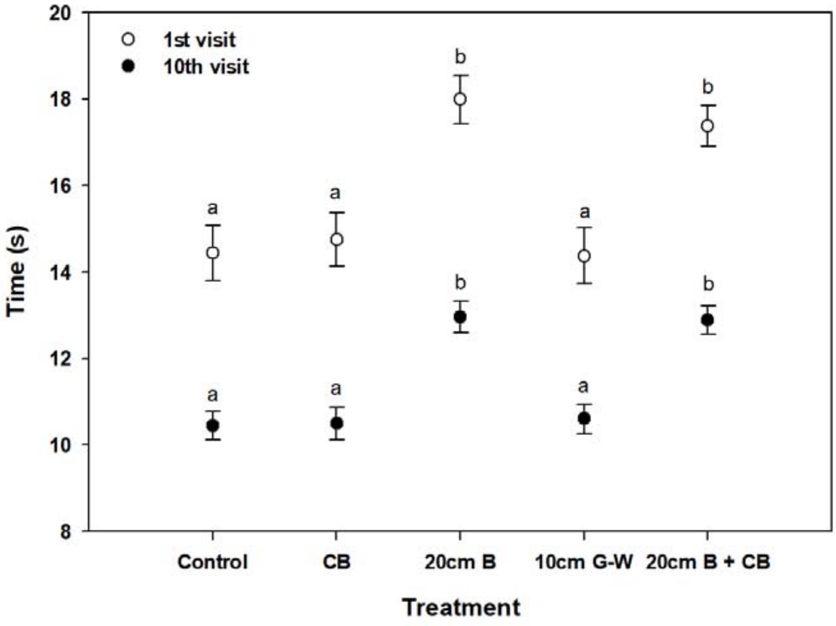
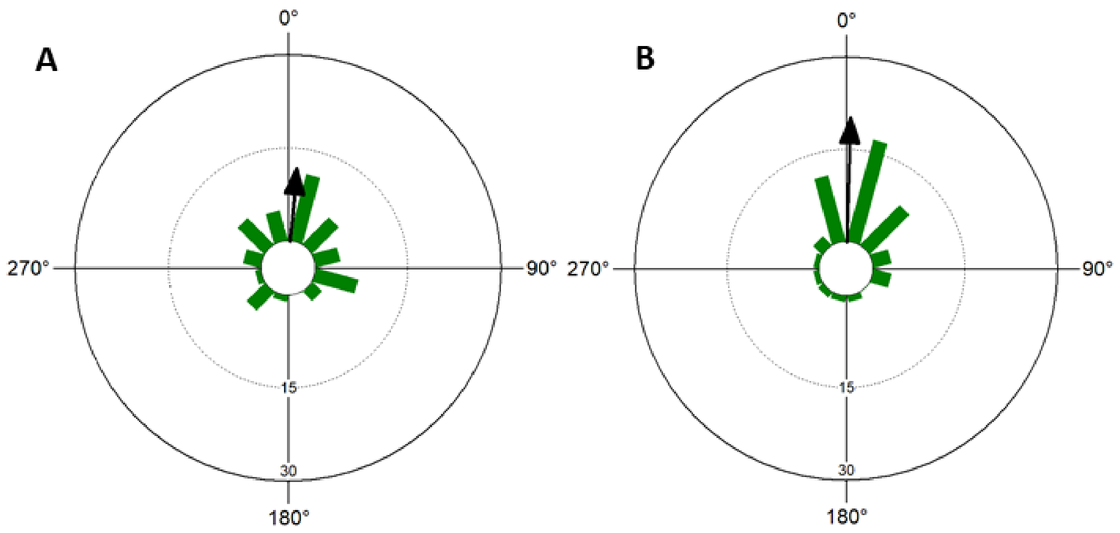


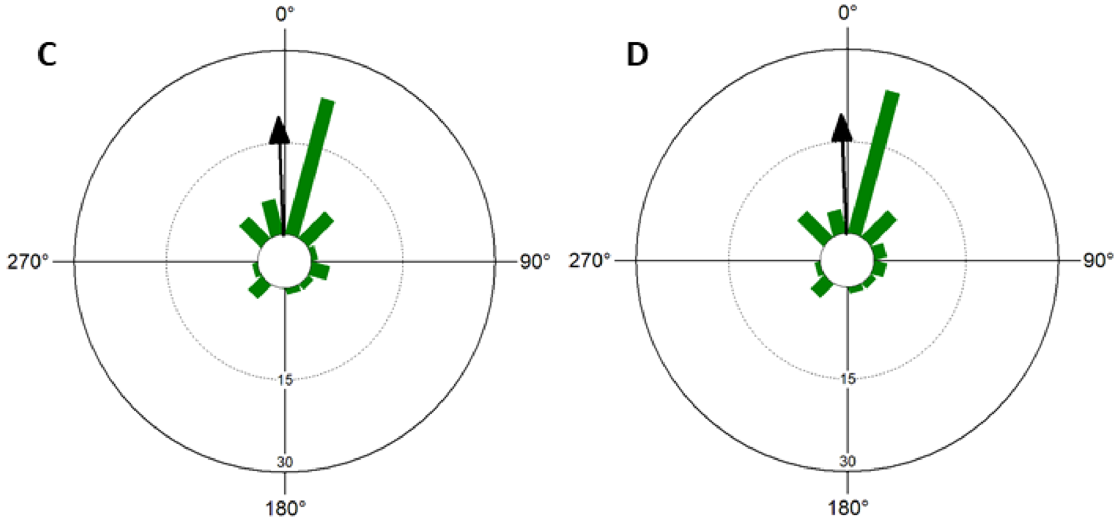
Figure 3: Mean time (mean ± s.e.m.) of foragers displacement from the release point to the limit of the goniometer (30 cm). Time considering the first (empty circles) and the last (filled circles) visits, as well as treatments. Different letters represent statistically significant differences ($p < 0.001$). CB = canopy blocked, 20cm B = Wall of 20 cm blocking the panoramic view, 10cm G-W = 10 cm of Gap-Wall, 20cm B+ CB = both panoramic view and canopy blocked.

In the analysis of differences between treatments regarding the homing ability of ants, we found difference in the orientation of ants tested in the control group at both distances of 15 and 30 cm (Fig. 4A-D), both in the first (Fig. 4A-B) and last (Fig. 4C-D) trips. At both distances, the orientation distribution was significantly non-uniform (Rayleigh test = $p < 0.05$). In addition, the angle direction of ants, was grouped around the mean considering the nest direction (V-test = $p < 0.05$).



15 cm (1st trip)
μ = 4.77°
r = 0.39
$p < 0.01$
V test < 0.01

30 cm (1st trip)
μ = 1.71°
r = 0.68
$p < 0.01$
V test < 0.01

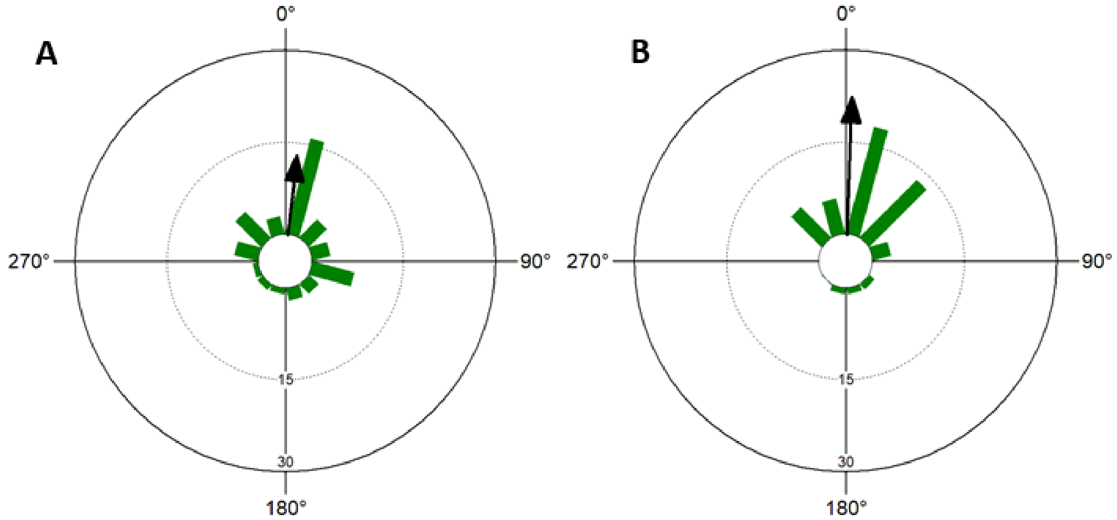


15 cm (10th trip)
μ = 357.37°
r = 0.64
$p < 0.01$
V test < 0.01

30 cm (10th trip)
μ = 357.40°
r = 0.65
$p < 0.01$
V test < 0.01

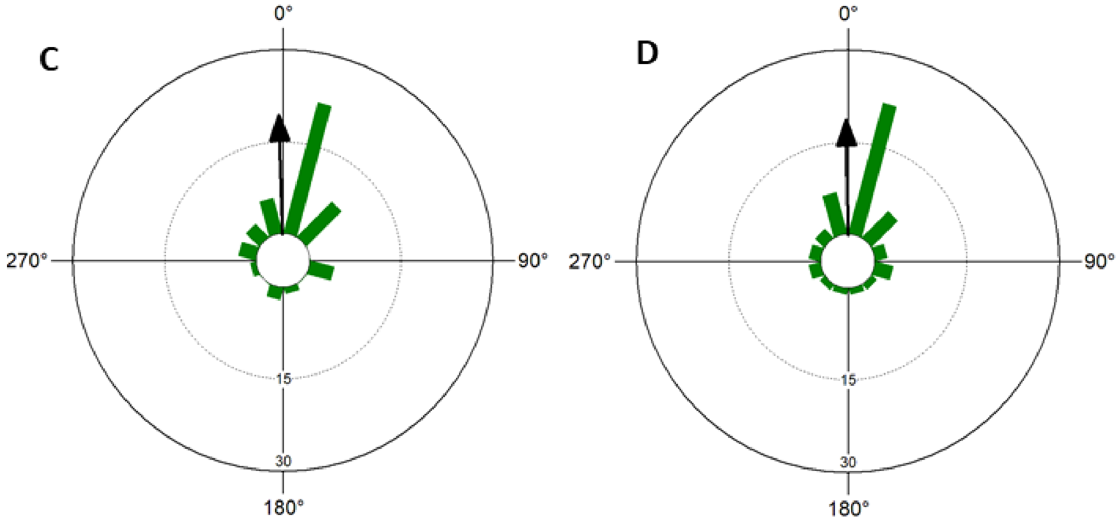
Figure 4: Circular analyses of ant behaviour during control treatment. The circular histograms show the mean direction (***μ***) and vector length (***r***) – represented by the arrow – of ants released in the centre of the goniometer when crossing the lines of 15 cm (A and C) and 30 cm (B and D), during the first (A and B) and last (C and D) trips, when all the environmental visual cues were available.

Both treatments of canopy block and partial block of the visual panorama (10 cm gap-wall) did not influence the behaviour of homing ants (Rayleigh test = $p < 0.05$), since they correctly oriented at both distances of 15 and 30 cm, as well as during the first and last trials (Figures 5 and 6) to the nest direction (V-test = $p < 0.05$).



15 cm (1st trip)
$\mu = 6.03°$
$r = 0.43$
$p < 0.01$
V test < 0.01

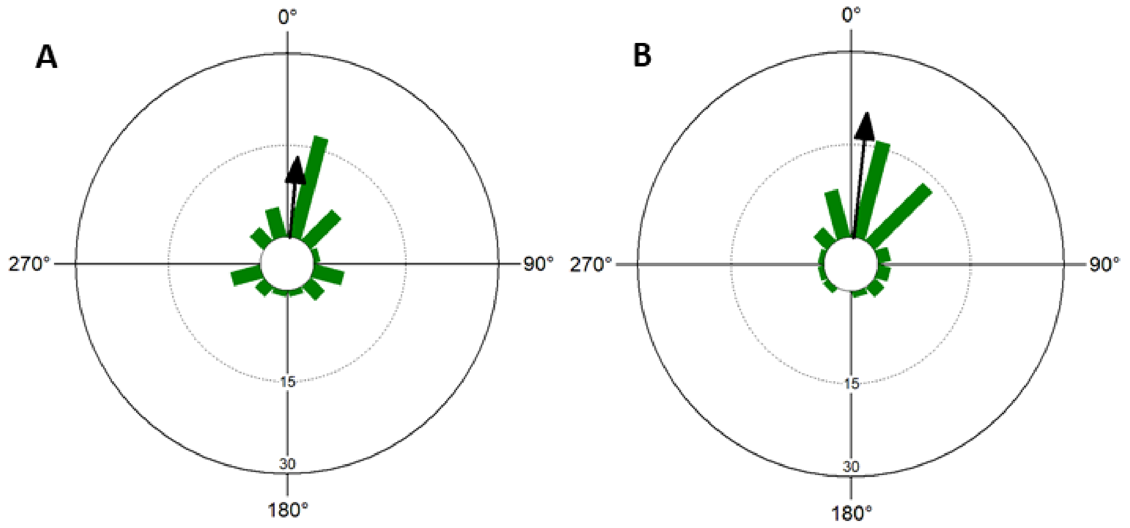
30 cm (1st trip)
$\mu = 2.17°$
$r = 0.75$
$p < 0.01$
V test < 0.01



15 cm (10th trip)
$\mu = 358.28°$
$r = 0.65$
$p < 0.01$
V test < 0.01

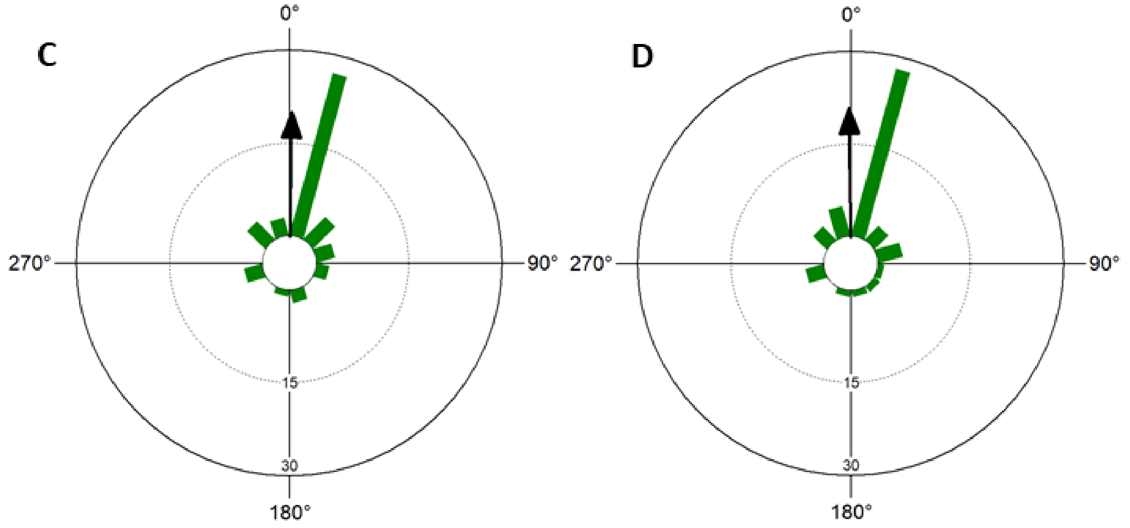
30 cm (10th trip)
$\mu = 359.32°$
$r = 0.63$
$p < 0.01$
V test < 0.01

Figure 5: Circular analyses of ant behaviour during canopy blocked treatment. The circular histograms show the mean direction (***μ***) and vector length (***r***) – represented by the arrow – of ants released in the centre of the goniometer when crossing the lines of 15 cm (A and C) and 30 cm (B and D), during the first (A and B) and last (C and D) trips, when the canopy was blocked, but panoramic visual cues were available.



15 cm (1st trip)
μ = 5.33°
r = 0.44
p < 0.01
V test < 0.01

30 cm (1st trip)
μ = 5.97°
r = 0.68
p < 0.01
V test < 0.01

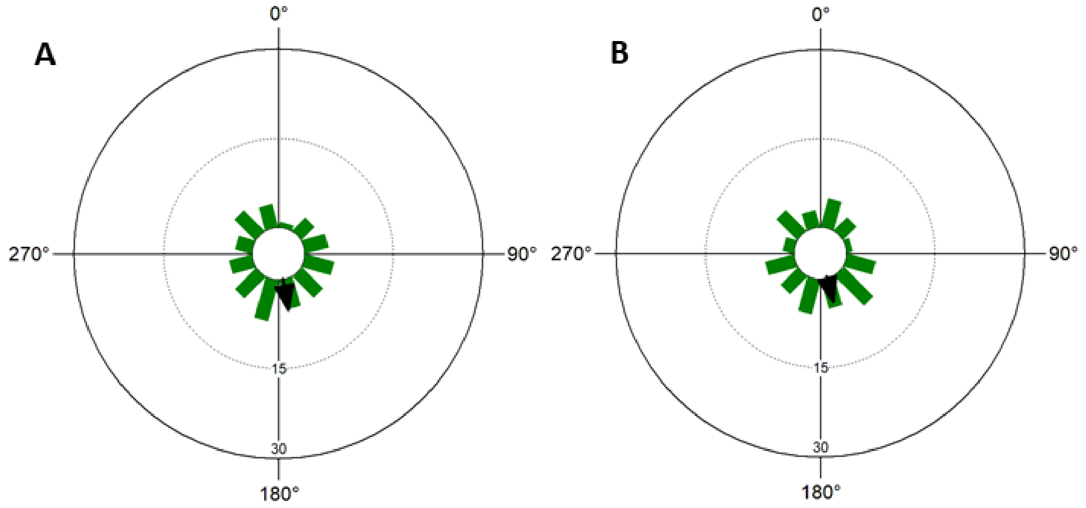


15 cm (10th trip)
μ = 0.92°
r = 0.67
p < 0.01
V test < 0.01

30 cm (10th trip)
μ = 359.34°
r = 0.70
p < 0.01
V test < 0.01

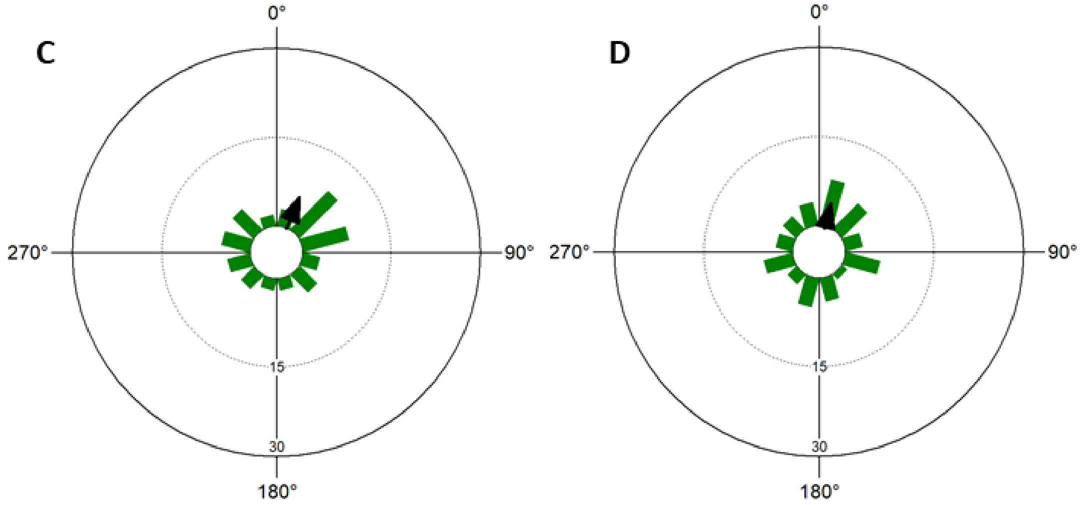
Figure 6: Circular analyses of ant behaviour during 10 cm Gap-Wall treatment. The circular histograms show the mean direction (***μ***) and vector length (***r***) – represented by the arrow – of ants released in the centre of the goniometer when crossing the lines of 15 cm (A and C) and 30 cm (B and D), during the first (A and B) and last (C and D) trips, when the panoramic view was partially blocked. The 10 cm gap was above the goniometer floor.

In contrast, the treatments of total block of the panoramic view (20 cm Wall) alone, or in association with canopy block (Wall of 20 cm + Canopy Block) interfered in the orientation of ants towards their nests (Figure 7 and 8). For these treatments, the Rayleigh test showed a clear uniformity in the orientation distribution of individuals in both 15 and 30 cm, and during the first and last trips. In these cases, V-test is meaningful, thus we did not perform this analysis.



15 cm (1st trip)
$\mu = 170.10^\circ$
$r = 0.17$
$p = 0.22$

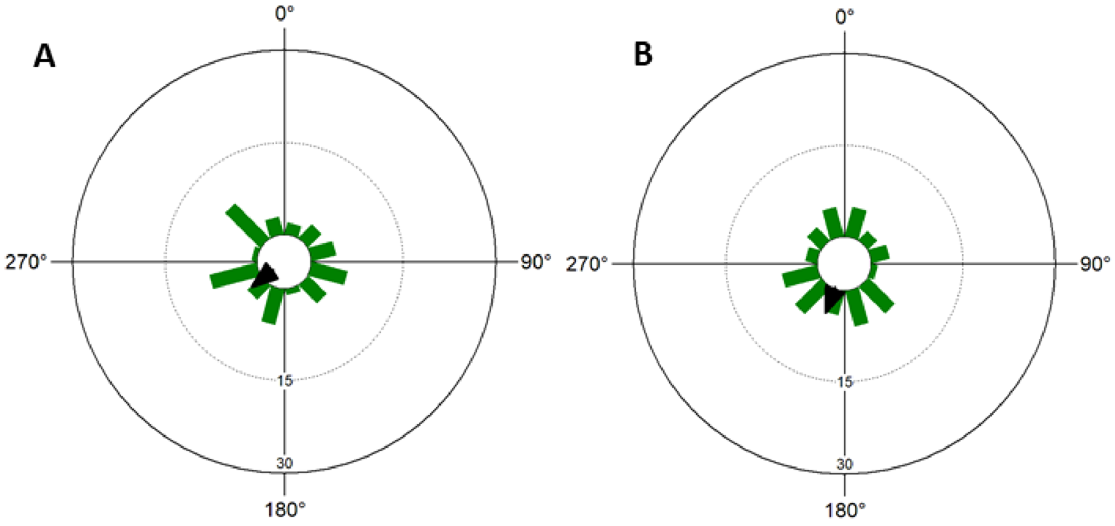
30 cm (1st trip)
$\mu = 165^\circ$
$r = 0.14$
$p = 0.40$



15 cm (10th trip)
$\mu = 22.47^\circ$
$r = 0.19$
$p = 0.16$

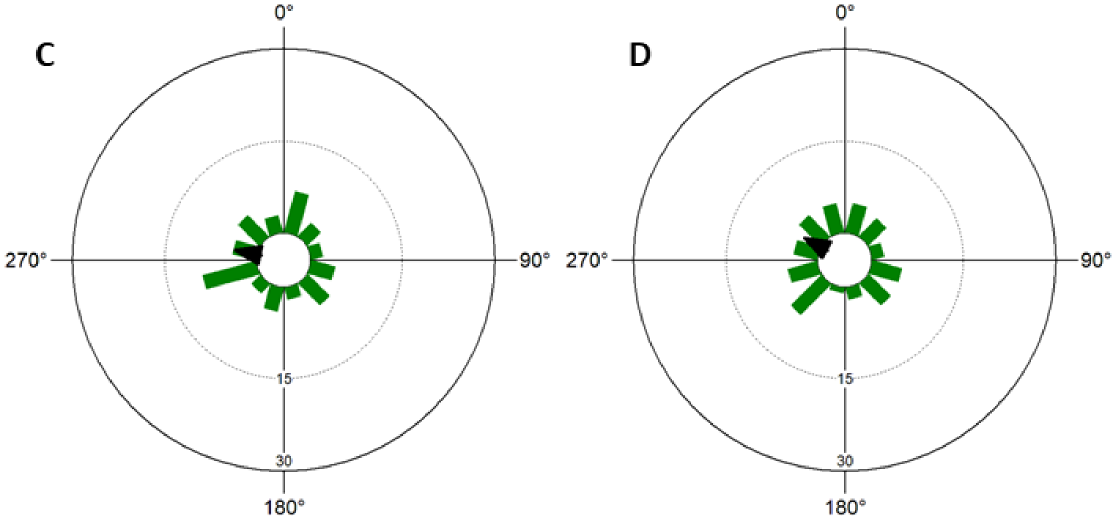
30 cm (10th trip)
$\mu = 13.36^\circ$
$r = 0.13$
$p = 0.40$

Figure 7: Circular analyses of ant behaviour during 20 cm Wall treatment. The circular histograms show the mean direction (***μ***) and vector length (***r***) – represented by the arrow – of ants released in the centre of the goniometer when crossing the lines of 15 cm (A and C) and 30 cm (B and D), during the first (A and B) and last (C and D) trips, when the panoramic view was completely blocked.



15 cm (1st trip)
$\mu = 232.24°$
$r = 0.07$
p = 0.75

30 cm (1st trip)
$\mu = 201.55°$
$r = 0.14$
p = 0.38



15 cm (10th trip)
$\mu = 281.48°$
$r = 0.12$
p = 0.45

30 cm (10th trip)
$\mu = 298.53°$
$r = 0.11$
p = 0.57

Figure 8: Circular analyses of ant behaviour during 20 cm Wall + Canopy blocked treatment. The circular histograms show the mean direction (***μ***) and vector length (***r***) – represented by the arrow – of ants released in the centre of the goniometer when crossing the lines of 15 cm (A and C) and 30 cm (B and D), during the first (A and B) and last (C and D) trips, when the horizontal panoramic view plus the canopy were blocked.

2.4. Conclusiones y valoración de la ejecución

The aim of this study was to begin to understand how ants use natural scenes for navigation inside complex environments, such as the Amazon forest. We tested the ability of *Gigantiops destructor* foragers to orient towards their nests when different portions of the visual scene were obstructed. We have shown that ants use visual information from the panoramic view to guide themselves, ignoring any possible cues from the canopy, even when panoramic information is completely obstructed (20 cm wall treatment). Although the use of path integration information is common among many different ant species (Collett & Collett, 2000), mostly those foraging in deserts environments, where visual landmarks are scarce, there is no evidence that during our experiments *G. destructor* was homing based on this information. Zero-vector ants did not show directionality towards the nest when deprived of canopy and panoramic scene (Figs. 7 and 8). Ants were faster and accurate in control, canopy blocked and 10cm Gap-Wall treatments to cross the 30 cm line, which indicates that they had enough visual information to correctly homing at this distance.

When the panoramic view is partially blocked (10 cm Gap-Wall treatment), *Gigantiops destructor* could homing by using the lower portion of the panorama available, showing that it was sufficient for visually guided orientation. Although this is impressive, a test blocking the lower part of the visual scene and allowing the upper part to be visible would tell us whether this capacity is exclusive of lower visual scenes or ants can also remember and extract information from the upper part. Unfortunately, we didn't perform such treatment. Given the results of the 10 cm Gap-Wall experiment, we felt the need to analyse images regarding the visual panoramic scene ants must be dealing with, from different routes and different nest backgrounds, to corroborate that nest direction truly differ from the rest of its near and far surroundings.

The acquisition of the 360 degrees camera, together with the tripod and automatic camera turner, was to analyse 360° panoramic images from the release points and routes of different individuals and nests. Ants have a visual field close to 360°, so these images will help us to understand what key visual information might be extracted from the panoramic scene when it is partially blocked (10 cm Gap-Wall). These images will receive a simple treatment and will be analysed in terms of rotational IDFs (i.e., Image Difference as a Function of the rotation) (Wystrach, Beugnon, & Cheng, 2011). Panoramic images can be rotated until they produce the best matching to the reference image (nets direction). Besides that, we still have to analyse the videos to access the time ants took to cross the 15 cm line and compare these results with those of 30 cm. Also, we are interested in understanding whether the choice of ants was more accurate during the first and last trials, at both 15 cm and 30 cm points. To that, we can simply calculate the absolute angle away from the group mean and perform a pairwise test.

In conclusion I can say that the aims of the project were achieved. We still have some complementary work to do and a final trip to the field area has been planned on March. Ants responded very well to our manipulations and experimental conditions, reducing the number of flights initially planned (five). Also, because I have established collaboration with Dr. Jorge and his student, they helped in the field work along the months, reducing even more the costs. Part of the stipend that was supposed to be invested in flights was invested in the purchase of new material (which possibility has been previously consulted with the secretary of AEET), aiming to better understand the visual system of *G. destructor*. As mentioned before, they present the largest eyes of any ant species and do not use pheromones or scent marks, which makes them an extreme interesting model for studies of visual ecology. Thus, I have been preparing a setup that consists in monochromatic LED lights, supported by a rechargeable battery in the field. I have faced some initial problems such as the specificity of the light (the description of providers usually didn't match the wavelength peak), their bandwidths, etc, but I believe now I could start a pilot of this new experiment during my visit on March. The general idea is to training ants to associate the reward with a light (different groups of ants will be trained with different lights, from 300 to 700 nm) to later test whether ants can discriminate the trained light from a novel one, and how good they are discriminating close wavelengths. The results of this experiment will let us to understand first, (a) which photoreceptors might be present in the eyes of *G. destructor* and second, (b) whether their visual capacity allows then to make colour discrimination between and within wavelength ranges. Many ants tested so far regarding their visual system has been demonstrated to be dichromat, but given the ecology of *G. destructor*, I expect this species to be trichromatic (three different types of photoreceptors). This result would partially help to explain the ability of this ant to navigate in complex environments without the use of chemical cues. Indeed, every ant tested during navigation experiments were preserved in order to have their eyes measured in the future to understand how their image resolution would be.

2.5. Publicaciones resultantes

Specifically, from the navigation results I expect to publish one paper till the end of this year. The novelty and quality of our data will allow us to try a scientific journal with a good impact factor such as Animal Behaviour or Journal of Experimental Biology. From the second idea regarding colour vision, I also expect one further publication, but given the specificity of the topic (visual system of one ant species), perhaps Journal of Comparative Physiology A would be a suitable option.

3. **Informe de gastos del proyecto.** Relación de partidas de gastos y sus importes. Se deberán aportar justificantes originales de los pagos realizados (tickets, recibos o facturas).

Unfortunately, I have to apologise because part of the costs cannot be justified. I have lost the receipts of subsistence costs during the field work (number in red in the table below). I am not sure what AEET will decide regarding this and the money that is left (which I was planning to use to buy the tickets of my last trip). In any case, I definitely can refund if it is necessary. I am more than glad for the grant and opportunity that I had, and all fruitful results from it.

Descripción	**(R$)**	**(€)**	**(US$)**
Car rental: first field trip	399.00		
Electronic related materials for the LED system (receipt Leroy Merlin)	253.67		
Tickets UDI-MAO-BELEN-UDI	1578.78		
Goniometer (before finding the most suitable size for ants in the field, I order two different sizes)	500.00		
LED lights from Germany (receipt leds_AlemaniaA and PI 131695B)		173.75	
Tripod + 360° timer (receipt Amazon.es - 403-4549938-2540354)		42.59	
Luces LED Ali Express 1			10.18

Luces LED Ali Express 2			6.68
Luces LED Ali Express 3			3.92
Luces LED Ali Express 4			5.05
Subsistence for the student helping with experiments during field work		400.00	
360° camera (factura cámara) + Protection cover (albarán carcasa)		430.67	
Density filters (receipt PrintOrder-E-2007779)		126.00	
LEDs holders (the value-added-tax of 19% in Germany because they did the bill for Brazil. Therefore, I also attached the “paypalExcerptB” that shows the full 113.53 EUR; receipt leds_113.53EURA)		113.53	
Power inverter 1000w 220v para 12v (Inversor de corriente)	149.90		
Rechargeable battery 12v 12a Moura (Bateria)	161.78		
Cable for battery charge 12v hasta 150a Original (cargador bateria)	(52.49 + 26.91 envío) 79.4		
Oriana software (de libras a euro = conversión 1.15)		65.97	

	R$ 3122.53	€ 1352.51	US$ 19.15
Conversión R$ para € = 0.23; Conversión US$ para € = 0.88	**€** 718.1819	**€** 1352.51	**€** 16.852
Total, gastos en Euros	2087.5439		
Total de la ayuda	2500		
Total disponible	**412.4561**		