

# Nutritional needs and mortality risk combine to shape foraging decisions in ants

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## Abstract

When foraging, internal needs for particular nutrients might affect food choice, and external constraints, such as predation risk, might impact trade-offs between foraging and risk avoidance. Examining both internal and external constraints simultaneously can provide important insights into how animals make decisions. We examined how internal nutritional needs and external cues of mortality risk jointly impact the foraging behavior of ants. Ant colonies require carbohydrates to support workers energetically and proteins to raise brood. Furthermore, colonies adjust their foraging activity in response to the environment, such as food availability and the presence of predators or heterospecifics. Here we examine the foraging decisions of groups of Argentine ants *Linepithema humile*, which differ in their nutritional needs in high-risk environments. We starved groups of ants for either proteins or carbohydrates and determined the foraging choices that ants made when cues of heterospecifics were present. We found that ants preferentially forage for carbohydrates in high-risk conditions. Furthermore, starvation for carbohydrates increased the ants' preference for carbohydrates, even when cues of heterospecifics were present at both carbohydrates and protein resources. Starvation for protein also resulted in preferential foraging for carbohydrates, but it increased visitation to a protein food source in high-risk environments compared to when ants were starved for carbohydrates or for both resources. Examining the effect of both nutrition and mortality risk on foraging simultaneously provides insights about state-dependent risk-taking behavior that may have important implications for predicting the invasion of species into novel habitats.

**Key words:** decision-making, foraging, nutrition, risk, trade-offs.

Animals constantly make decisions based on different, sometimes conflicting, needs while facing a range of constraints on their behavior. The effects of constraints on animals' behavior are often studied independently of one another (Hendriksma and Shafir 2016; Cheh et al. 2021; Hayes et al. 2021). However, an examination of multiple constraints simultaneously can provide important insights into the priorities that animals make when optimizing multiple factors simultaneously (Sih et al. 1990). For example, multiple factors influence foraging decisions, including nutritional needs and predation risk (Houston et al. 1993). While the impact of mortality risk on foraging and the nutritional needs of animals have both been examined separately to explain foraging decisions, the combination of these 2 factors is important to investigate together to gain a thorough understanding of how animals behave in natural situations (Moran et al. 2020).

The level of risk in the environment (e.g., the presence of predators and competitors) can impact foraging success. Individuals may experience novel and unknown environments and can become susceptible to predation while foraging for food, leading to trade-offs between spending time foraging versus exploring new environments and being vigilant for predators (Lima and Dill 1990). While foraging, being watchful of predators can lead to decreased food intake (Krebs 1980) and can impact optimal foraging decisions (Stephens and Krebs 1986). Indeed, some animals spend less time foraging

when predation risk is present (Verdolin 2006; Liesenjohann and Eccard 2008). Furthermore, different types and levels of risk may lead to different effects on animal's choosiness among food types (McArthur et al. 2014; Charalabidis et al. 2017), can determine the persistence of behavioral responses (Luttbeg and Sih 2010; Wolf and Weissing 2010; Toscano et al. 2016), and can impact ecological communities by altering the nature of interactions among species with different trade-offs (Lebrun and Feener 2007).

The physiological condition of an animal can influence its likelihood to forage in high-risk environments. The “asset protection principle” (Clark 1994) suggests that animals in good physiological condition are less likely to take risks because their condition is important to protect. Conversely, the “state-dependent safety hypothesis” (reviewed in Moran et al. 2020) proposes that individuals in good condition are more likely to take risks because they have a higher likelihood to survive such risks—due to their good physiological conditions. Furthermore, state-dependent risk-taking models (Barclay et al. 2018) show that animals with higher physiological needs (e.g., caused by not finding food) are expected to take greater risks in the presence of predators (McNamara and Houston 1986). For example, in graybelly salamanders, *Eurycea multiplicata griseogaster*, satiated individuals took the longest amount of time to attack prey in the presence of a predator and hungry individuals took the shortest amount

of time to attack prey in the absence of a predator (Whitham and Mathis 2000).

Animals require an array of nutrient types and their needs for particular nutrients may shape their foraging decisions (Simpson and Raubenheimer 2012). For example, animals need both carbohydrates and proteins and alter their foraging decisions according to their needs (Vaudo et al. 2016). These nutritional needs change over time as ecological and physiological conditions change and can impact morphology (Hawlena et al. 2011). When animals produce offspring, for instance, they require different nutrients than when they migrate (Bromley and Jarvis 1993).

The foraging of ants is influenced by environmental conditions and nutritional needs. Ants abandon high-risk environments when provided with low-risk options (Nonacs and Dill 1988; Kay and Rissing 2005) and forage more frequently at low-risk sites (Nonacs and Dill 1988). Ants also balance their foraging behavior, adjusting the intake of carbohydrates and proteins, based on the nutritional needs of the colony (Dussutour and Simpson 2008, and 2009). For example, fire ants recruit, that is, communicate to their nest mates about food and lead them to it, twice as strongly to sugar compared with protein when deficient in carbohydrates; however, they switch to recruiting to protein once they have consumed a sufficient amount of sugar (Cassill and Tschinkel 1999). However, the role of mortality risk in foraging decisions that distinguish between different nutrients is unknown.

We use Argentine ants *Linepithema humile* as a model system for examining the joint effects of nutritional needs and the level of mortality risk in the environment on foraging decisions. *Linepithema humile* are an invasive species throughout the world (Suarez et al. 2001). One reason for their successful displacement of native species is their greater foraging efficiency compared to native species, with Argentine ants finding and exploiting food faster than native species (McGrannachan and Lester 2013). *Linepithema humile* forage for both carbohydrates (e.g., honeydew from aphids and scale insects) and proteins (e.g., dead animals) because workers rely on carbohydrates as an energy source, and protein is needed for larval growth (Markin 1970). Depending on the needs for each nutrient, foraging behaviors can potentially be affected. Groups of *L. humile* prefer low-risk foraging sites, but will also forage in high-risk locations (Lessig and Nonacs 2021). Furthermore, whether or not an individual has recently fed influences its foraging behavior in high-risk environments. Satiated individuals (with full gasters) are more likely to return to the nest in high-risk environments (e.g., when alarm cues are present), and workers whose gasters are not full linger near the threat (Halley and Elgar 2001) consistent with the “asset protection principle” (Clark 1994) and contrary to the “state-dependent safety hypothesis” (Moran et al. 2020). This response to mortality risk might be beneficial to the colony because satiated workers are more valuable in the immediate future than hungry ones because they supply the colony with resources (Nonacs and Dill 1990). However, it is not known whether deprivation of particular nutrients influences the decisions of *L. humile* groups to forage at sites with high risk. Understanding whether or not *L. humile* forage in high-risk environments when faced with different nutritional needs can uncover the ways in which the invasive *L. humile* successfully establish themselves and flourish in nonnative environments, especially considering the impact of carbohydrate supply on the success of their invasion (Rowels and Silverman 2009)

and aggression (Grover et al 2007) and, more broadly, the importance of different behaviors for the spread of invasive species (Chapple et al. 2012; Rehage et al 2016).

Here we examine how nutritional needs influence the foraging behavior of *L. humile* in high-risk environments. We first hypothesize that starvation for all nutrients will affect a group’s decision to forage in a high-risk environment. We predict that, like individual ants (Halley and Elgar 2001), starved groups will be more likely to forage at high-risk sites compared to groups that were not starved. Furthermore, we examine how starvation for particular nutrients influences the group’s foraging decision in a high-risk environment. We hypothesize that the type of nutrient that the group is starved for will determine which nutrient it forages for in a high-risk environment. Specifically, we expect groups deprived of carbohydrates to forage preferentially at a site with carbohydrate-rich food as opposed to a site with protein-rich food if both represent a high-risk environment. Similarly, we expect groups that are starved for protein to forage preferentially at high-risk sites with protein-rich food and mortality risk cues compared to high-risk sites with carbohydrate-rich food (Table 1).

## Materials and Methods

### Collection and maintenance of ants

We collected *L. humile* workers from foraging trails and nest entrances at 3 different locations in the UCLA Botanical Gardens and near the Life Science Building throughout June–July 2020 and April–June 2021. The first records of invasive *L. humile* in southern California date back to early last century (Woodworth, 1908). We collected ants from different foraging trails at the botanical gardens, which were within 200 m of each other. Before setting up an experiment, we collected approximately 900 workers from a single location and brought them to the lab. Three hundred ants were housed in a single cylindrical plastic cup (diameter = 90 mm, height = 108 mm), with sides coated in fluon to prevent the escape of the ants. We refer to these cups as the “housing area/cup” and we allowed ants to acclimate to it for 48 h before experiments began. The acclimation period began as soon as the ants were brought into the lab. Inside the housing cup, we provided the ants with 4 culture tubes (diameter = 12 mm, length = 75 mm): 2 filled with water blocked by a cotton ball to provide a constant water supply and 2 with a damp cotton ball at its bottom and wrapped in aluminum foil to form a dark cavity, as a nest for the workers (Figure 1, similar to Neumann and Pinter-Wollman 2022).

To test food preference, we connected the housing cup by plastic tubing (inside diameter = 12 mm, length = 185 mm [approximate]) to 2 other cups of the same dimension as the housing cup, which acted as the foraging locations during the experiment (Figure 1). We prevented ants from entering the tubes for the 48-h acclimation period by plugging the tube entrances with cotton balls. Each foraging cup consisted of either a protein-rich or carbohydrate-rich food, following a recipe from Dussutour and Simpson (2008) and using the ingredients detailed in Table 2. For the preparation of the different foods, the agar and boiling water were mixed and allowed to sit for approximately 5 min to activate the gelatinous properties of the agar. The remaining ingredients were then mixed in a glass container and combined with the agar and water solution. This mixture was then placed in the

**Table 1** Treatments carried out to test our hypotheses

Treatment	Carbohydrates <sup>1</sup>	Protein <sup>1</sup>	Mortality risk cues <sup>2</sup>	Hypothesis tested	Prediction	Findings in this study
1 No starvation, low risk	+	+	–	Starvation for any nutrient will affect a group's decision to forage in a high-risk environment—when mortality risk cue is present	Less foraging activity compared to treatment #3 and more foraging than #2	Did not find evidence to support this prediction
2 No starvation, high risk	+	+	+		Less foraging activity compared to treatments #4 and #1	Found evidence supporting the opposite of this prediction
3 Starvation for both, low risk	–	–	–		Greater foraging activity compared to treatments #1 and #4	Did not find evidence to support the first part of this prediction but found support for the second part
4 Starvation for both, high risk	–	–	+		Greater foraging activity compared to treatment #2 and less than #3	Found evidence supporting the opposite of this prediction
5 Starved for carbohydrates, high risk	–	+	+	The type of nutrient that the group is deficient in will determine its decision on what to forage in a high-risk environment—when a mortality risk cue is present.	Greater foraging on carbohydrates compared to protein when mortality risk cues are present at both sites, and compared to #4	Strong evidence
6 Starved for protein, high risk	+	–	+		Greater foraging on protein over carbohydrates when mortality risk cues are present at both sites, and compared to #4	Partial evidence—groups starved for protein have a slight preference for carbohydrates over protein early in the experiment, however, protein foraging of ants starved for protein is highest compared to all other treatments.

<sup>1</sup> In these two columns, a + indicates that the ants received the nutrient before the experiment and a – sign indicates that they were starved for that nutrient.

<sup>2</sup> In this column, a + indicates a mortality risk cue was present at both food sites during the experiment and a – sign indicates that a mortality risk cue was not present at either food source.

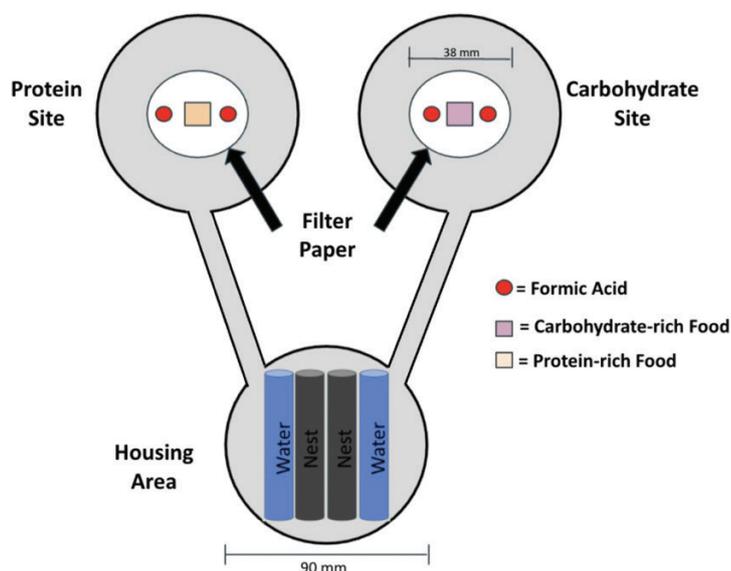
refrigerator where it solidified and was stored for further use. At the end of each experiment, ants remained in the lab and were cared for until they died naturally, usually within a few weeks from the end of the experiment. Ants are invertebrates and so do not require institutional animal care protocols for experimentation; however, we took extreme care not to harm the ants when conducting our work.

### Experimental procedure

To determine how mortality risk influences the food preference of ants with different nutritional needs we set up 6 different treatments. Each treatment was replicated 6 times, with 6 unique groups of 300 ants in 2020. Each group experienced only 1 treatment. One control (no starve, low risk) was added in 2021 and had only 3 replicates with 500 ants in each group, and 2 more treatments (no starvation, high risk and starve for both, high risk) were repeated 3 more times with 500 workers in each replicate in 2021. We could not repeat the other treatments in 2021 due to the unexplained death of ants. The order of treatments was determined haphazardly and the date on which each trial was conducted is included in the statistical model as a random effect to account for the potential impact of when a treatment was conducted on the results.

To determine food preference, we provided the 2 different food types, carbohydrate rich and protein rich, 1 in each of

the 2 foraging areas (Figure 1) during the experiments detailed below. The food was provided as a small chunk (approximately 1 cm<sup>3</sup>) placed on circular pieces of filter paper (diameter = 38 mm [approximately]) in the center of the foraging areas (Figure 1). To test the effect of mortality risk, we placed formic acid near the food. Formic acid was used because it is a mechanism for defense by formicine ant species against other species of ants, including *L. humile*, and because of its lethality, it has been used previously as a mortality risk cue to study *L. humile* risk-taking behavior (Lessig 2019) and was shown to be a more effective mortality risk cue than live conspecifics in a foraging context (Lessig and Nonacs 2021). We placed 2 drops of formic acid (95% concentrated), using a standard eye drop syringe on a circular piece of filter paper (diameter = 38 mm [approximately]) on both sides of the food (Figure 1). We used this amount of formic acid because we found in preliminary trials that a larger amount could kill the ants. Furthermore, we left the lids of the foraging areas open to allow ventilation and prevent death from overexposure to formic acid, which dissipated at a slow-enough rate to remain effective until the end of the experiment. Ants were allowed access to the food areas by removing the cotton plugs from the tubes at the start of the experiment and we counted the number of ants at the food, as detailed below. We provided enough food for it to not be depleted during the experiment.



**Figure 1** Schematic (A) and photo (B) of the experimental setup with a housing area/cup connected to 2 foraging areas with plastic tubes (approximately 185 mm length). In 1 foraging area, we provided protein-rich food and in the other carbohydrate-rich food. (A) White circles in the food areas are the filter paper, on which we placed the food (orange and pink squares) and drops of formic acid (red dots). The housing area included 2 test tubes with water and 2 test tubes covered with foil as a nest.

**Table 2** Food composition

Food Type	Whey Protein Concentrate (g)	Calcium Caseinate (g)	Whole Egg Powder (g)	Sucrose (g)	Water (mL)	Agar (g)
Protein-rich	2.33	2.33	0.98	1.88	37.50	0.50
Carbohydrate-rich	0.45	0.45	0.98	5.63	37.50	0.50

Quantities of the different ingredients in the 2 types of nutrients provided in the experiments: protein-rich and carbohydrate-rich (adapted from [Dussoutour and Simpson 2008](#)).

To test the effect of nutritional needs on food preference in high-risk environments, we varied whether ants were starved or not for both or 1 of the 2 nutrient types. We conducted 1 of the 6 treatments below by providing (or depriving) particular nutrient types in the housing area during the 48-h acclimation period ([Figure 1](#)). Ants were never allowed into the foraging areas during the 48-h acclimation period; food provided during the acclimation period (if ants were not starved) was given directly in the housing area.

- 1. No starvation, low risk**—To control for the effect of starvation and the effect of mortality risk cues, we provided the ants with both nutrient types (carbohydrates and protein) during the 48-h acclimation period and did not include a mortality risk cue (formic acid) at either food site when testing for food preference.
- 2. No starvation, high risk**—To control for the effect of starvation, we provided the ants with both nutrient types (carbohydrates and protein) during the 48-h acclimation period. When testing for food preference, both food sites included a mortality risk cue.
- 3. Starvation for both, low-risk**—To control for the effect of mortality risk cue at the food sites, we starved the ants during the 48-h acclimation period and then allowed them to forage at the 2 food sites without mortality risk cues near the food when testing for food preference.

- 4. Starvation for both, high-risk**—To test the effect of starvation on foraging in high-risk environment, we deprived the ants of both proteins and carbohydrates during the 48-h acclimation period. When testing for food preference, both food sites included a mortality risk cue.
- 5. Starvation for carbohydrates, high-risk**—To test the effect of starvation for carbohydrates on ants' food preference in high-risk environments, we starved the ants during the 48-h acclimation period only for carbohydrates, that is, the protein-rich food was available to them *ad lib* during acclimation. When testing for food preference, both food sites included a mortality risk cue.
- 6. Starvation for protein, high-risk**—To test the effect of starvation for protein on ants' food preference in a high-risk environment, we starved the ants during the 48-h acclimation period only for protein, that is, the carbohydrate-rich food was available to them *ad lib* during acclimation. When testing for food preference, both food sites included a mortality risk cue.

#### Quantifying ants at food

To determine the number of ants at carbohydrates or proteins in each of the 6 treatments and 6 replicates, we counted the number of ants anywhere in each foraging area (including on the filter paper) every 5 min for 1 h. We determined that an ant was in a foraging area if it was anywhere in the foraging

**Table 3** Output of the GLMM—analysis of deviance table

	Chi <sup>2</sup> ( $\chi^2$ )	df	P-value
Treatment	2,249.802	5	< 0.0001***
Site visited (protein/carbohydrates)	88.556	1	< 0.0001***
Time (within the trial)	2,444.982	1	< 0.0001***
Treatment × Site visited (protein/carbohydrates)	447.660	5	< 0.0001***
Treatment × Time	213.529	5	< 0.0001***
Site visited (protein/carbohydrates) × Time	41.554	1	< 0.0001***
Treatment × Site visited (protein/carbohydrates) × Time	87.865	5	< 0.0001***

\*\*\* Indicates statistically significance results

area and had completely exited the tube leading from the housing area such that its entire body was inside the foraging area. After the recording period ended, we removed all the ants from the experimental apparatus (housing and foraging areas) and cleaned it entirely with ethanol.

### Data analysis

To determine the effect of both mortality risk cues and nutritional needs on foraging behavior, we used a generalized linear mixed model (GLMM) with a Poisson link function for count data using the “lme4” R package (Bates et al. 2015). The number of ants at food was the response variable and the explanatory variables included: experimental treatment (see list of 6 treatments above), food visited (carbohydrates or protein), and time (as a continuous variable within each trial), we further included all interactions among these fixed effects. To account for potential dependencies among counts within a trial, we included “replicate” as a random effect, and to account for an effect of either year (and therefore group size—because 1 year we used 300 ants and the other we used 500 ants) and the date on which each treatment was conducted, we included “year” and “date” as random effects. To compute the conditional and marginal  $R^2$  values we used the R package “MuMIn” (Barton 2020). For specific comparisons across treatments, and to determine whether ants preferred 1 resource over the other, we used post hoc Tukey tests using the “emmeans” R package (Lenth 2022). First, we used the post hoc tests to determine the effect of risk level on foraging by starved groups (comparing treatments 3 and 4 in Table 1), and the effect of risk level on foraging by satiated groups (comparing treatments 1 and 2 in Table 1). Then, to determine the effect of starvation on foraging in low-risk environments, we used a post hoc test to compare treatments 1–3, and in high-risk environment, we used a post hoc test to compare treatments 2–4 listed in Table 1. Finally, we used a post hoc test to compare the number of ants visiting carbohydrates and proteins for treatments 5 and 6 in Table 1. We used R version 4.2.2 (R Core Team 2022) for all analyses and plotting. Data and code are available in Supplementary Materials.

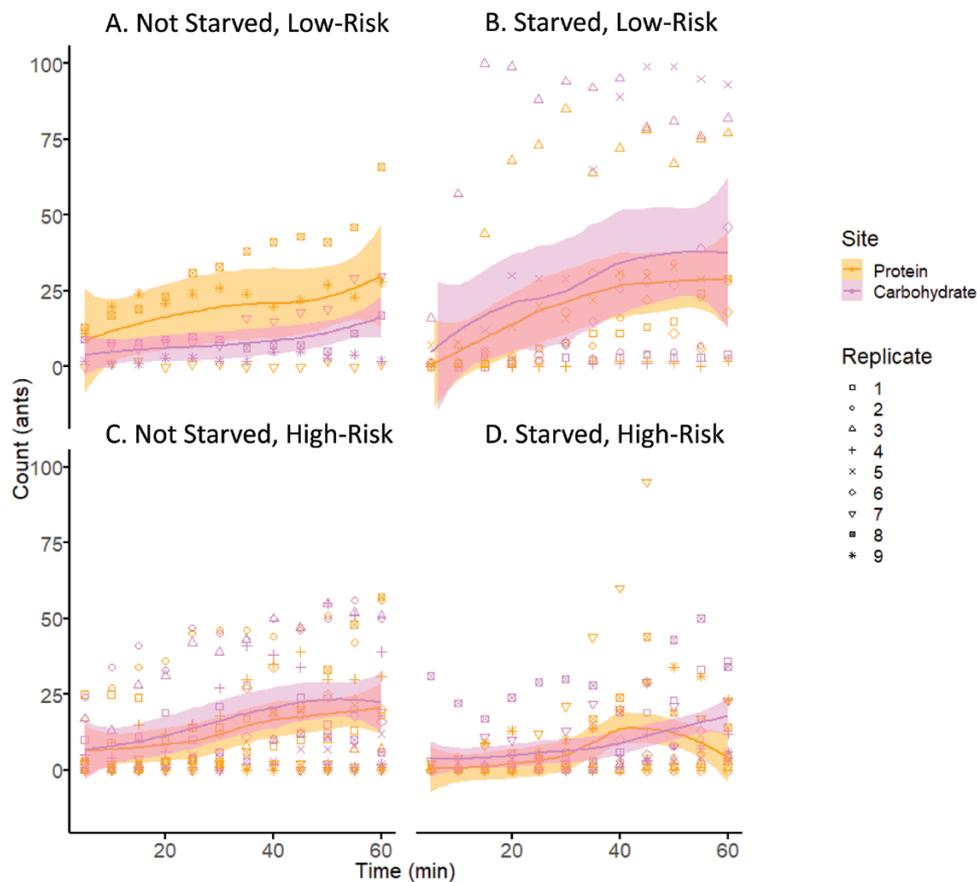
### Results

We found that nutritional deficiencies and the presence of mortality risk cues both impacted the foraging decisions of *L. humile* groups. First, as expected in a species with recruitment to food, the number of ants that visited the carbohydrates or protein food sources increased over time throughout the 60 min of the experiment (GLMM: Time: Estimate +  $SD$  = 0.017 + 0.002;  $\chi^2$  = 2444.982,  $df$  = 1,  $P$  < 0.0001, Table 3,

Figures 2 and 3). Second, ants visited carbohydrates significantly more than protein (GLMM: Site visited (protein/carbohydrates): Estimate +  $SD$  = 1.093 + 0.176;  $\chi^2$  = 88.556,  $df$  = 1,  $P$  < 0.0001, post hoc Tukey test: ratio = 0.76,  $SE$  = 0.019,  $P$  < 0.0001, Table 3, Figures 2 and 3). Third, starvation and mortality risk cues affected the number of ants at the food sites (GLMM: Treatment:  $\chi^2$  = 2249.802,  $df$  = 5,  $P$  < 0.0001, Table 3, Figures 2 and 3; specific post hoc comparisons below). The conditional  $R^2$  of the model was 0.97 and the marginal  $R^2$  was 0.34. All 3 random effects had a very small contribution to the variance of the model: replicate (variance +  $SD$  =  $2 \times 10^{-9}$  +  $4.5 \times 10^{-5}$ ), year (variance +  $SD$  =  $5.5 \times 10^{-10}$  +  $2.3 \times 10^{-5}$ ), and date of experiment (variance +  $SD$  = 1.7 + 1.3).

Nutritional deficiencies affected the groups’ foraging decisions in high-risk environments. Treatments differed in whether ants preferred carbohydrates or protein (GLMM: Treatment × Site visited (protein/carbohydrates):  $\chi^2$  = 447.66,  $df$  = 5,  $P$  < 0.0001, Table 3, Figures 2 and 3) and in how quickly ants were recruited to the food (GLMM: Treatment × Time:  $\chi^2$  = 213.529,  $df$  = 5,  $P$  < 0.0001, Table 3, Figures 2 and 3). Furthermore, there was a difference in how quickly ants were recruited to carbohydrates or protein (GLMM: Site visited (protein/carbohydrates) × Time:  $\chi^2$  = 41.554,  $df$  = 1,  $P$  < 0.0001, Table 3, Figures 2 and 3). Recruitment to carbohydrates or protein differed across treatments (GLMM: Treatment × Site visited (protein/carbohydrates) × Time:  $\chi^2$  = 87.865,  $df$  = 5,  $P$  < 0.0001, Table 3, Figures 2 and 3). Risk level affected foraging by starved groups (comparing treatments 3 and 4 in Table 1): Starved groups recruited more individuals in low-risk environments compared to high-risk environments (post hoc Tukey test: ratio = 5.839,  $SE$  = 0.257,  $P$  < 0.0001, Figure 2B,D). However, ants that were not starved did not seem to differentiate between high- and low-risk environments (comparing treatments 1 and 2 in Table 1; post hoc Tukey test: ratio = 0.97,  $SE$  = 0.55,  $P$  = 0.995, Figure 2A,C). When there was no mortality risk cue, starved and not starved groups (treatments 1 and 3) did not significantly differ in their foraging (post hoc Tukey test: ratio = 0.895,  $SE$  = 0.056,  $P$  = 0.483, Figure 2A,B). Interestingly, contrary to our prediction, when groups were starved, they were less likely to forage at sites with mortality risk cues, compared to groups that were not starved (comparing treatments 2 and 4; post hoc Tukey test: ratio = 5.385,  $SE$  = 0.2374,  $P$  < 0.0001, Figures 2C,D).

In line with our predictions, we found that groups that were deprived of a particular resource tended to forage more readily on the resource they were deprived of in high-risk environments compared to when they were starved for both, and mortality risk cues were present, for both types of



**Figure 2.** Number of ants over time (every 5 min for 1 h) at the foraging site where a protein-rich (orange) or carbohydrate-rich (purple) food was provided. Treatments included low-risk (A, B) or high-risk (C, D) environments (mortality risk cue absent/present at both food sites) and ants being starved for both resources (B, D) or not starved for either (A, C). Relating these plots to the treatments in Table 1: A-1, B-3, C-2, D-4. Each replicate in each treatment is represented as a different shape. Solid lines represent the smoothed average of the counts from the different replicates and the shaded area is the 95% confidence interval, both produced using the `geom_smooth()` function in the “ggplot2” (Wickham 2016) package of R, with the default Loess method.

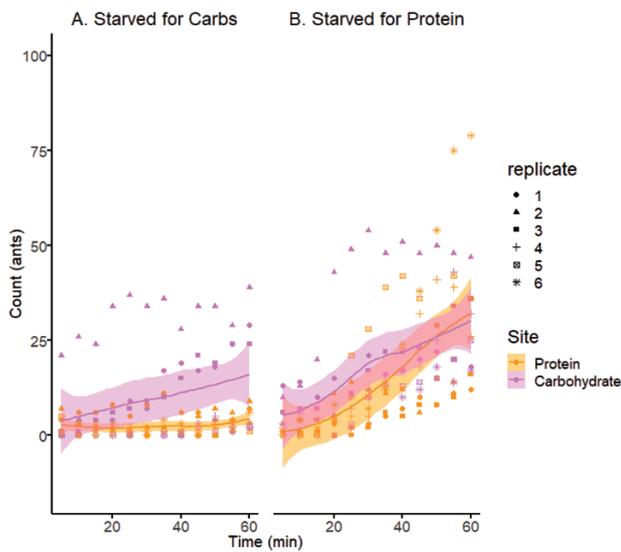
resource (post hoc Tukey test: starved for carbohydrates vs. starved for both, high risk: ratio = 1.574,  $SE = 0.0925$ ,  $P < 0.0001$ ; starved for protein vs. starved for both, high risk: ratio = 3.092,  $SE = 0.143$ ,  $P < 0.0001$ ; Figure 3 vs. Figure 2D). Groups deprived of carbohydrates tended to forage less at high-risk sites with protein relative to high-risk sites with carbohydrates (post hoc Tukey test: ratio = 0.273,  $SE = 0.0234$ ,  $P < 0.0001$ , Figure 3A). Similarly, groups deprived of protein tended to forage less at high-risk sites with protein relative to high-risk sites with carbohydrates (post hoc Tukey test: ratio = 0.636,  $SE = 0.033$ ,  $P < 0.0001$ , Figure 3B). Finally, when ants were starved for protein, more individuals visited the high-risk protein food source compared to when ants were starved for carbohydrates (post hoc Tukey test: ratio (c/p) = 0.334,  $SE = 0.2919$ ,  $P < 0.0001$ , Figure 3 [orange in both panels]) and compared to groups that were starved for both resources and were presented with mortality risk cues at both food sites (post hoc Tukey test: ratio = 2.667,  $SE = 0.176$ ,  $P < 0.0001$ , orange in Figure 3B vs. Figure 2D).

## Discussion

We found that nutritional needs influenced the foraging decisions of *L. humile* groups in high-risk environments. We found strong support for our hypothesis that the type of nutrient that ants are deprived of will determine their

foraging decisions in high-risk environments. When ants were starved only for carbohydrates, they tended to forage more on carbohydrates with mortality risk cues relative to protein with mortality risk cues (Figure 3A). In addition, when ants were starved only for protein, their foraging on protein with mortality risk cues was higher than their foraging on protein in any other treatment in which mortality risk cues were present (Figures 2 and 3), even though they had a slight preference for carbohydrates over protein early in the experiment, when both were present (Figure 3B). However, we did not find evidence supporting our prediction that starvation for both nutrients would affect a group’s decision to forage in high-risk environments, when mortality risk cues are present. When there were no mortality risk cues, we did not find a difference between the foraging activity of ants that were starved or not (Figure 2A,B). When mortality risk cues were present, ants that were not starved tended to forage more on food with mortality risk cues compared to starved ants, contrary to our predictions (Figure 2C,D).

We expected starved groups to forage at high-risk sites more readily than groups that were not starved, but we found the opposite (Figure 2C,D). Previous modeling (McNamara and Houston 1986; Clark 1994) and empirical (Skutelsky 1996; Sandhu et al. 2018) work predicted that hunger would lead to a greater likelihood to forage in high-risk environments because hungry animals have less to lose than satiated



**Figure 3** Number of ants over time (every 5 min for 1 h) at the foraging site where a protein-rich (orange) or a carbohydrate-rich (purple) food was provided for the treatments in which mortality risk cues were present at both sites and ants were starved for either carbohydrates (A, treatment 5) or proteins (B, treatment 6) before the experiment. Each replicate in each treatment is represented as a different shape. Solid lines represent the smoothed average of the counts from the different replicates and the shaded area is the 95% confidence interval, both produced using the `geom_smooth()` function in the “`ggplot2`” (Wickham 2016) package of R, with the default Loess method.

individuals, also known as the “asset protection principle” (Clark 1994). However, our findings contrast with these predictions and are consistent with the “state-dependent safety hypothesis” (Moran et al 2020), which proposes that individuals in good condition are more likely to take risks because they have a higher likelihood to survive those risks. Thus, our findings suggest that certain factors, such as activity level and energetic costs of starvation, are important for *L. humile* foraging decisions. Furthermore, it is possible that temporal variation in mortality risk, which we did not examine here, could impact the collective foraging of *L. humile*, according to the “predation risk allocation” hypothesis (Lima and Bednekoff 1999), which proposes that animals change their behavior in response to changes in the risk level associated with the behavior (in this case foraging). Finally, it is possible that the ants in the starved treatment of our study simply did not have enough energy to forage. Future work could examine the energetic costs of starvation and identify the physiological limits that prevent starved animals from being able to forage in high-risk environments.

When groups were starved for carbohydrates they preferred to feed on carbohydrate-rich foods over protein-rich foods in high-risk environments, as we predicted (Figure 3). This finding suggests that when a particular resource is scarce, the benefits of obtaining it outweigh the potential costs imposed by a high-risk environment. While deficiencies in carbohydrates increase foraging for this resource in other social insects (bumble bees: Hendriksma et al. 2019; *Dorymyrmex* ants: Kay 2004), it was unknown previously whether such food preferences will emerge in high-risk environments. Our findings that a strong preference for foraging on carbohydrates persists when starved for this nutrient and in high-risk environments highlight the importance of carbohydrates to ants.

While the order in which recruiting ants find a food source may influence which food they utilize, in more than half of the experiments the ants found both food sources at the same 5 min observation period. Furthermore, when 1 food type was found first, it did not necessarily end up being the preferred food by the end of the experiment (tested using a logistic regression—see analysis code in the [Supplementary Materials](#)). Future work could examine the level of risk above which ants that are starved for carbohydrates no longer forage for carbohydrates to fine-tune our understanding of the costs and benefits of foraging for particular nutrients.

We found partial support for the prediction that when ant groups are starved for proteins they will prefer protein-rich foods in high-risk environments (Figures 2 and 3). While we found a preference for carbohydrate-rich food when groups were starved for protein, this preference was more apparent early in the experiment than later (Figure 3B), suggesting that if we had run the experiment for longer a preference for protein might have emerged. Moreover, groups that were starved for protein foraged on protein in high-risk environments more than the foraging for protein seen than in any other treatment (comparing the orange data in Figure 3B to the orange data in all other figures was statistically significant in post hoc Tukey tests). Argentine ant workers require carbohydrates as an energy source and proteins are used for feeding brood (Markin 1970). The groups in our experiment had only workers without brood, which might have led to the lack of preference for protein in the protein-starved treatment. Future work comparing the foraging decisions of groups with and without brood, might reveal a stronger preference for protein-rich foods in high-risk environments when brood is present. Furthermore, protein-rich foods reduce the longevity of ants (Dussutour and Simpson 2012), thus it is possible that the negative impacts of proteins on ant physiology may result in a higher threshold for preferring protein over other nutrients. Still, the increase in recruitment to protein when starved for it that we observed, even in the absence of brood, suggests that recent exposure (or lack of exposure) to particular nutrients can impact foraging decisions. Indeed, other invertebrates, including ground beetles *Agonum dorsale*, wolf spiders *Pardosa prativaga*, and desert spiders *Stegodyphus lineatus* regulate their protein intake based on their previous nutritional experience—after being deprived of protein they increased its intake (Mayntz et al. 2005). A wide range of vertebrate carnivores hunt particular prey or eat specific body parts that are rich in the desired macronutrient, such as proteins and lipids (Kohl et al. 2015). Future studies may examine which physiological and social conditions result in a preference for protein over other nutrients that goes beyond an overall increase in recruitment to protein.

To conclude, making foraging decisions in high-risk environments can depend on nutritional needs. Although starved animals may be more likely to forage in high-risk environments than satiated individuals (Moran et al 2020), high levels of starvation may reduce the likelihood of foraging in high-risk conditions, perhaps because of high energetic costs. Furthermore, nutrients differ in their importance, with starvation for some nutrients resulting in a greater likelihood of foraging in high-risk environments compared to starvation for other nutrients. Finally, it is important to examine both changes in preference as well as changes in overall foraging activity as distinct measures of effects on foraging behavior. Examining the responses of animals that experience different

nutritional backgrounds in environments with different levels of risk allows us to uncover the intricacies of the trade-offs that animals face.

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## Author Contributions

BB conceived the study and performed all the experiments. BB and NPW designed the experiments, analyzed the data, and wrote the manuscript jointly.

## Supplementary Material

Supplementary material can be found at <https://doi.org/10.1093/cz/zoac089>.

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