Received: 4 September 2025 | Accepted: 28 July 2026

DOI: 10.1111/1365-2656.70337

Journal of Animal Ecology

RESEARCH ARTICLE

Effects of heat stress on the foraging activity of incipient ant colonies

Rafael Carvalho da Silva | Maëlys Prost | Angélique Bultelle | Thibaud Monnin

Sorbonne Université, Université Paris Cité, Université Paris Est Créteil, CNRS, INRAE, IRD, Institut d'Écologie et des Sciences de l'Environnement de Paris (UMR7618), Paris, France

Correspondence
Rafael Carvalho da Silva
Email: rcswasp@gmail.com

Funding information
French National Research Agency (ANR) under the France 2030 programme, Grant/Award Number: ANR-22-EXES-0004

Handling Editor: Tom Bishop

Abstract

1. Global warming affects organismal performance across biological levels, from molecular processes to complex behaviours. Ectotherms are particularly vulnerable to thermal stress, as their body temperature critically depends on ambient environmental conditions. Warming may affect their foraging behaviour and/or their activity patterns. Here, we experimentally investigated the effects of acute and chronic thermal stresses on foraging by an ectotherm species.
2. Social insects are important ecosystem engineers. Colony survival and growth depend on successful foraging, and colonies are more susceptible to warming when they are young and have fewer workers. We investigate how heat intensity and exposure duration influence foraging dynamics and survivorship in young colonies of the *Lasius niger* ant.
3. Using laboratory assays, we studied the effects of acute heat stress by exposing foragers to short (10 min) and intermediate (1 h) heat pulses of 25°C (control), 30°C, 35°C and 40°C. Furthermore, we investigated the effects of chronic heat stress by rearing colonies at 25°C:20°C (day:night, control conditions) or 35°C:30°C (heat stress) for 2 weeks. After heat exposure, we recorded the latency to initiate foraging, the number of foraging visits and individual and colony survival between treatments.
4. We hypothesized that ants could cope with relatively low and relatively short heat shocks, but not with high or long heat exposures. We found that prolonged and high heat exposure indeed increased the mortality of foragers and colonies, increased the delay before foraging, and decreased the number of foraging trips.
5. Reduced foraging capacity in young colonies is likely to have strong fitness consequences. The establishment of colonies is dependent on the rapid acquisition of resources to sustain exponential growth. Any slowdown in resource intake can limit colony development and prolong the duration of the incipient (newly founded) and vulnerable stage, ultimately reducing the likelihood of successful establishment.

KEYWORDS
foraging, global warming, heat stress, *Lasius niger*, social insects, thermal accomodation

1 | INTRODUCTION

Global climate change shapes species distribution, adaptive responses, and survival strategies around the world (Bellard et al., 2012; Lister & Garcia, 2018; Urban, 2015). Although global warming affects all organisms, ectotherms may be particularly vulnerable, as their body temperatures are largely determined by ambient conditions (Huey & Kingsolver, 1989; Kingsolver et al., 2013; Seebacher et al., 2015). This dependence can lead to different outcomes depending on body size: smaller organisms tend to exhibit higher thermal tolerance compared with larger ones, but their survival rates may decline more rapidly over time (Peralta-Maraver & Rezende, 2021). Insect larvae developing at higher temperatures grow faster and emerge as overall smaller adult insects or with smaller specific structures, which may affect their physiology, thermal resistance, and interactions with the environment (Guiraud et al., 2021; Moradinour et al., 2023). This may be particularly relevant during early colony development in social insects, when the first workers are typically smaller than those produced later. Because body size itself can be an important determinant of individual thermal sensitivity (Nascimento et al., 2022), such effects can eventually scale up to influence colony-level performance. Although many ectotherms exhibit a degree of plasticity that confers some thermal resilience, environmental temperatures can push them towards or even beyond their thermal tolerance limits (Kingsolver et al., 2013; Sunday et al., 2014). In this context, thermal stress can influence patterns of resource acquisition and foraging behaviour in ectotherms (Gérard et al., 2024; Vollet-Neto et al., 2015).

Thermal stress is determined by both the intensity of heat and the duration of exposure, as formalized in the concepts of thermal death time (TDT) and thermal load sensitivity (TLS). The former describes how the intensity and duration of exposure determine lethal limits, and the latter incorporates non-lethal effects and accounts for damage repair and acclimation processes (Arnold et al., 2025; Jørgensen et al., 2019; Rezende et al., 2014). By experimentally varying heat intensity and exposure duration, we can test whether behavioural responses depend jointly on temperature and exposure time, as predicted by time-dependent thermal stress frameworks.

Across taxa, elevated temperatures affect feeding and foraging, including timing, resource preference, speed, and efficiency, and often force trade-offs between energy acquisition and thermal safety (Angilletta Jr, 2009). Social insects exhibit a variety of collective traits that can buffer thermal stress, including modification of nest architecture (García Ibarra et al., 2024; Sankovitz & Purcell, 2021) and resource storage (Menzel & Feldmeyer, 2021). Most colony members (i.e., queen, brood, and non-foraging workers) do not leave the protection of the nest (cool environment, water, and food availability). Foragers cannot avoid exposure to stressing environmental conditions, but they can adjust foraging strategies to minimize exposure (e.g., foraging in cooler periods). A recent example is the *Bombus terrestris bumblebee*, which exhibits changes in foraging behaviour under warmer conditions (24°C vs. 32°C), including a preference for collecting water over sugar solutions (Gérard et al., 2025), as well as a reduced flower handling time coupled with increased visitation rates and flight speed (Gérard et al., 2024). Additionally, foragers may benefit from the nest environment and resources when recovering from heat damage.

Ants provide a compelling system to study these dynamics, as they are directly exposed to environmental conditions, for example, increased soil temperature while foraging (Parr & Bishop, 2022). Furthermore, given their strong influence as ecosystem engineers and their capacity to modify their surroundings, understanding the impact of increasing temperatures on their activities is of considerable ecological relevance (de Souza & Delabie, 2021; Parr & Bishop, 2022).

In ants, foraging is critical to colony survival and growth, with important implications for the evolution of morphology, behaviour, and physiology (Carroll & Janzen, 1973). Elevated temperatures can significantly alter foraging dynamics (Cerdá et al., 1998; Roeder et al., 2021; Roeder et al., 2022), although responses vary between species and environments. The meat ant *Iridomyrmex purpureus* reduces activity at high temperatures (above 50°C), while maintaining relatively stable running speeds (Andrew et al., 2013). In tropical ecosystems, species differ markedly in their sensitivity to thermal conditions. *Atta colombica* and *Azteca trigona* easily resume foraging when heat stress is removed, while *Cephalotes atratus* and *Dolichoderus bispinosus* remain deterred by localized heat exposure such as sunflecks (Spicer et al., 2017) (but see also Roeder et al., 2022, where temperature improves foraging performance in *Pogonomyrmex barbatus*).

The effects of high temperatures on foraging dynamics may be especially concerning for incipient (newly founded) ant colonies. In these early stages, nutritional demands are met by a limited workforce, whose output may be less resilient to strong perturbations such as a warmer environment (Hölldobler & Wilson, 1990; Peeters & Ito, 2001; Porter & Tschinkel, 1986). In addition, in many species, the first generation of workers is small (often referred to as 'nanitic'). In ant species with polymorphic workers, small workers often have lower heat resistance than larger conspecific workers (see, e.g., Nascimento et al., 2022; Arnan et al., 2022). Thus, incipient colonies with few and nanitic workers may be particularly susceptible to heat. Despite the ecological relevance of colony performance across different life stages and the increasing temperatures associated with environmental change, most studies addressing the impact of thermal stress on ant foraging activity have focused on workers already present on foraging trails in the field, without necessarily accounting for colony ontogeny (e.g., Spicer et al., 2017; Trigos-Peral et al., 2024). Although these approaches are pertinent, they may limit our ability to interpret how temperature shapes foraging dynamics, particularly during incipient phases.

By identifying this gap in the literature and inspired by some aspects of the TDT framework (e.g., the joint effects of temperature and exposure duration in determining thermal limits), our objective was to explicitly test the interaction between heat intensity and exposure duration on the foraging behaviour of incipient colonies of *Lasius niger* (L.). This species is a common European ant that

inhabits natural environments and open areas such as pastures, gardens, and roadsides, and is often abundant in urban environments (Czechowski, 2024; Czechowski et al., 2012). Mated queens found new colonies either alone or in small groups, but always without the support of workers, making this stage particularly vulnerable (Sommer & Hölldobler, 1995). The first generation of workers is nanitic and typically emerges within the first few weeks following nest initiation (personal observation; Madsen & Offenberg, 2017; Roux et al., 2026). We used the first generation of workers produced by queens of incipient colonies to test whether foragers would cope well with relatively low heat or relatively short heat shocks, but not with high heat or long heat exposures. Specifically, we predicted that colonies experiencing the latter conditions would exhibit reduced foraging performance, characterized by increased latency to initiate foraging and fewer foraging visits. By focusing on incipient colonies and the joint effects of heat intensity and duration of exposure, our study addresses a key dimension of thermal vulnerability shaping the foraging performance of early-stage social insect colonies. Given the foundational role of foraging in colony development, understanding how heat events affect this behaviour provides valuable insight into how episodic extreme heat events associated with global climate changes can disrupt population persistence from the earliest stages of the life cycle.

2 | MATERIALS AND METHODS

2.1 | Ant collection and rearing conditions

The mated queens (~400) were collected in Leuven, Belgium, in July 2024 immediately after their nuptial flight. They were reared at the laboratory in Paris. Each queen was placed in a test tube containing water ad libitum. The tubes were kept in climate-controlled chambers (CTS brand) under a temperature regime of 25°C:20°C (day:night, with 10 h:10 h plus 2 h of ramping up and down). After the emergence of the first workers, the colonies were fed weekly apple sauce and *Tenebrio molitor* larvae. In November 2024, the temperature was gradually lowered to 12°C (decrease of 1°C per day over 2 weeks) to simulate seasonal cooling. From December 2024 to February 2025, it was maintained at a constant 12°C. At the end of February, the temperature gradually increased to 25°C:20°C. The colonies were then transferred to plastic boxes containing tubes with ad libitum water and plaster as a substrate. They were allowed to acclimate to the new housing for at least 3 days prior to being used in foraging experiments. A total of 242 colonies were used in the experiment. Each experimental colony consisted of one queen, its first-generation nanitic workers, and the brood (eggs, larvae, and pupae). Because the sizes of the colonies naturally varied despite being reared under standardized conditions, we selected colonies with similar sizes (numbers of workers and larvae) to balance the treatments. Most colonies were precisely counted (counting the number of workers and larvae) immediately before being tested for foraging. At that time, they contained 12.99 ± 7.62 workers (range: 1–40) and 12.85 ± 7.73 larvae (range: 0–46) and were of similar size in most treatments (Figure S1). The colonies of one treatment (2 weeks at 35°C:30°C) were slightly less populous than those of the corresponding control (2 weeks at 25°C:20°C). This probably stems from a negative effect of this long-lasting treatment; that is, colonies presumably had a similar size at the onset of heat treatment, but controlled colonies may have grown more than stressed colonies. To test this, we randomly selected 49 additional colonies and submitted them to the same two treatments (chronic treatment at 25°C:20°C or 35°C:30°C), but this time we counted the number of ants at the beginning and end of the treatment. Further methodological details are provided in Section 2.3. No ethical approval was necessary to conduct the present study.

2.2 | Heat stress treatments

Heat stress treatments combined two exposure durations with four heat intensities, while a third exposure duration was tested with only two temperature intensities. We used exposure durations of 10 min, 1 h, and 2 weeks (hereafter referred to as short, intermediate, and chronic heat stress, respectively). Short and intermediate heat stress treatments were applied at 25°C (control), 30°C, 35°C, and 40°C, while chronic heat stress was applied in two temperature regimes: 25°C:20°C (control) and 35°C:30°C. Note that a 4 h exposure at 43°C induces high mortality in *L. niger* queens, which are much larger than nanitic workers (Roux et al., 2026). Short and intermediate exposures consisted of a heat shock applied to workers reared under non-stressful (control) conditions, whereas chronic exposures consisted of rearing entire colonies under stressful conditions, as detailed below.

For short and intermediate exposures, the queen and the brood were left in the colony under control rearing conditions (25°C:20°C), while the workers were gently collected from their colony using soft tweezers and placed on a high precision thermal plate (PZ60 from Präzitherm®—Precision hotplates, heating surface 200 × 280 mm) set at the target temperature of the heat shock. Thermal plates generate a thermal gradient, with the heat decreasing from their surface upward. This technique is well suited to our experiment, as it mimics natural conditions occurring in open habitats of temperate regions, where the soil is heated by direct sunlight and where temperature rapidly decreases upward. Alternative techniques such as water or dry baths, or climate-controlled chambers as we used for chronic stress (see below), do not produce a heat gradient but homogeneous heat coming from all directions, and may be better suited to study species from closed or tropical habitats. Although it is difficult to compare the thermal limits obtained using different methodologies (see, e.g., Corley et al., 2023), this was not the primary objective of our study. Instead, our aim was to investigate how heat stress affects foraging behaviour. The workers tested were distributed in 15 bottomless circular plastic tubes (5.2 cm in diameter × 6 cm in height, open at both ends) coated with Fluon® and placed on the thermal plate, already at the target temperature, to prevent them from escaping. After the assigned heat shock was completed, the workers

were immediately returned to their colony, the colony housing box was coupled with the foraging apparatus, and foraging measurements began. Most colonies used for short or intermediate exposures were used in two to three trials. These trials were spaced out by at least 10 days and assigned in random order. This was taken into account in the statistical analysis (see below). Note that only workers were treated and the 10-day delay between trials allowed them to fully recover from their previous heat exposure. In fact, ants from various subfamilies typically recover their locomotion ability from a heat coma in about 10 min (Leong et al., 2023), and *Drosophila* males thermally stressed during their development recover in 6 days (Canal Domenech & Fricke, 2022). A summary of colonies used more than once can be found in Table S1.

For chronic exposure, entire colonies were kept under the studied temperature regime in climate-controlled chambers for 2 weeks before foraging trials, that is, 25°C: 20°C (control) or 35°C: 30°C (high). Colonies assigned to chronic high stress (35°C:30°C) were only used once, as extended exposure probably introduced cumulative thermal stress, which would potentially confound the results. Some of the colonies assigned to the chronic control (25°C:20°C) were also used in short or intermediate exposures. This is not an incidence as the chronic control temperature is the standard rearing conditions. Preliminary tests showed that all founding queens reared under a 40°C:35°C regime died within a few days ($n = 60$), while those reared under 35°C:30°C and 25°C:20°C thrived. Consequently, no incipient colonies were exposed to chronic stress at 40°C:35°C, as it had proved to be lethal.

All colonies were deprived of food (but not water) for at least 4–5 days prior to testing to stimulate foraging. Treatments and number of replicates are summarized in Table 1.

2.3 | Mortality

For acute treatments, worker mortality was measured immediately after treatment (that is, by counting the number of workers who died on the thermal plate). For the chronic treatment, in which entire colonies were reared under specific temperature regimes, we recorded queen mortality by checking if they were alive or not just before the foraging experiment, after 2 weeks of treatment (all colonies).

We measured the number of workers alive in a subset of 49 colonies maintained for 2 weeks at 25°C:20°C ($n = 29$) or 35°C:30°C ($n = 20$), comparing the number of workers present on Day 1 versus Day 14. Furthermore, we continued to monitor queen mortality after completion of the 2-week chronic exposure and foraging test (at that time three queens had died in the 35°C:30°C treatment) to assess colony survival over time and to determine the maximum number of days they could withstand this chronic heat stress. This monitoring was stopped when the last colony in the stressed group (35°C:30°C) died.

2.4 | Foraging activity

Foraging activity was measured immediately after workers (for short and intermediate treatments) or colonies (for chronic treatment) had been exposed to heat stress. A 3D printed bridge (8 cm long) was connected to the colony nest box, providing access to a coverslip containing a 50% sucrose solution ad libitum. We video-recorded the bridge including the glass coverslip for 4 h, at 5 frames per second, using Python via the integrated development environment Thonny. Images were compiled into a video using Fiji software for subsequent manual analysis. The analysis of the videos was carried out with the treatment conditions

TABLE 1 Heat stress treatments and sample size.

Treatment	Rearing conditions (day:night)	Heat shock	Duration of heat exposure	Sample (number of colonies)
Acute short	25°C:20°C	25°C (control)	10 min	30
Acute short	25°C:20°C	30°C	10 min	29
Acute short	25°C:20°C	35°C	10 min	33
Acute short	25°C:20°C	40°C	10 min	31
Acute intermediate	25°C:20°C	25°C (control)	1 h	28
Acute intermediate	25°C:20°C	30°C	1 h	30
Acute intermediate	25°C:20°C	35°C	1 h	25
Acute intermediate	25°C:20°C	40°C	1 h	31
Chronic	25°C:20°C	(control)	2 weeks	41
Chronic	35°C:30°C		2 weeks	30 of 35[a]
Chronic	40°C:35°C		2 weeks	0 of 60 queens[b]

Note: Acute treatments consisted of a heat shock (short duration = 10 min; intermediate duration = 1 h) applied to the tested foragers only (otherwise raised under a control temperature regime). Chronic treatments consisted of rearing entire colonies under various temperature regimes for 2 weeks before testing foragers.

[a]35 colonies were established. Five died during the thermal exposure period and could not be used.

[b]60 solitary founding queens were set up. All died in less than a week.

(heat intensity and duration of exposure) hidden from the observer. A foraging event was recorded from the moment an ant arrived on the coverlip and stayed still. We recorded the delay before the onset of foraging (i.e., when the first worker visited the food source) and counted the number of foraging visits during the 4 h of video.

2.5 | Statistical analysis

All statistical analyses were performed with R (version 4.4.0) through RStudio (version 2025.09.2) (R Core Team, 2021). We analysed the effects of temperature (heat intensity and duration of exposure) on the delay before the onset of foraging, the number of foraging visits, and the mortality of queens as response variables. Furthermore, we inferred the mortality of workers by measuring the number of workers alive before and after temperature treatment. Day and colony identification were included as random effects to account for temporal variability and repeated measures. The count data were initially modelled using Poisson GLMMs; however, these showed signs of overdispersion and therefore were refitted using negative binomial GLMMs through the *glmmTMB* package (Brooks et al., 2017). Post hoc comparisons were performed using the *emmeans* package with Tukey correction.

The latency to initiate foraging and queen mortality after chronic exposure plus foraging phase were analysed using survival analysis. A Kaplan–Meier model was fitted to compare the time until the first foraging event (or queen death) between temperature treatments. The response variable was the time to first visit (in seconds) (or death of the queen), and colonies that had not initiated foraging within the observation window (or where the queen did not die) were treated as censored. The survival object was constructed using the surv(i) function of the *survival* package (Therneau, 2015), and the group-specific survival curves were fitted using survfit(ii). These were visualized with the ggsurvplot() function of the *survminer* package (Kassambara et al., 2016), including confidence intervals and logarithmic rank *p*-values.

Finally, we evaluated the survival of the queen for all colonies that underwent chronic exposure by comparing the percentage of queens alive versus dead in temperature treatments before and after chronic exposure using Fisher's exact test, implemented via the fisher.test() base R function. Fisher's test was also used to compare the percentage of nests in which we found at least one dead worker after exposure to thermal plates. Data handling and visualization

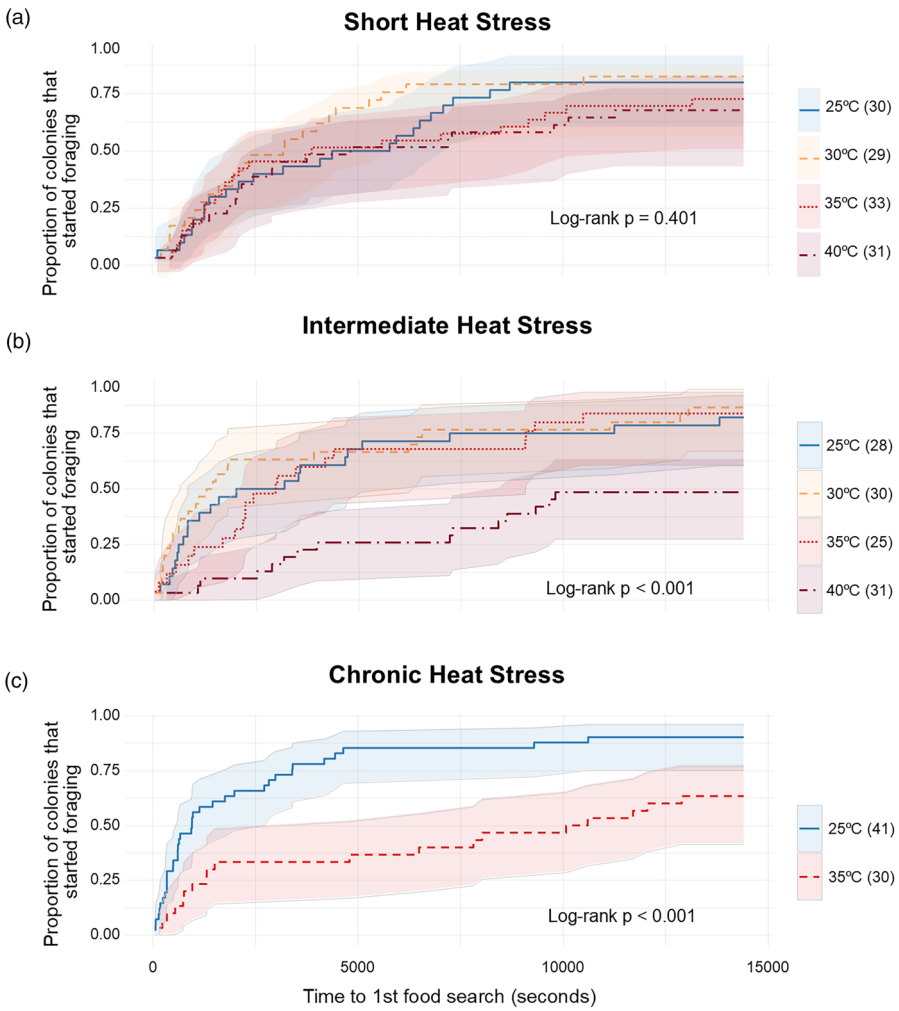

FIGURE 1 Delay before the onset of foraging. Survival curves represent the cumulative proportion of colonies for which foraging started over time for short (a), intermediate (b), and chronic (c) heat stresses. A steeper curve indicates a shorter delay, whereas flatter curves reflect a slower or less consistent response. The numbers of colonies tested are given in parentheses.

were supported by the packages *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2018).

3 | RESULTS

3.1 | Latency before foraging

The latency before the start of foraging (that is, before the first worker reached the sugar solution) was affected by both the duration and intensity of heat stress. Short heat stresses did not have an effect on the foraging latency, at least within the heat intensity range tested (25–40°C, log rank $p = 0.401$; Figure 1a). Intermediate-duration heat stresses delayed foraging, but only when they were of the highest heat intensity (40°C), with less intense stresses having no effect (25°C vs. 40°C: Log rank $p < 0.001$, $X^2 = 10.989$; 30°C vs. 40°C: p-value < 0.001, $X^2 = 15.624$; 35°C vs. 40°C: p-value < 0.001, $X^2 = 10.847$; Figure 1b). Lastly, chronic heat stress (rearing below 35°C:30°C) delayed foraging compared to control rearing conditions (25°C:20°C, Log rank $p < 0.001$, $X^2 = 13.200$; Figure 1c).

Comparison of delay according to duration of exposure within each temperature showed that most of the groups did not differ from each other (Figure S2). However, a small effect was detected between short and chronic exposures at 25°C ($X^2 = 6.446$, $p = 0.011$), where colonies exposed to chronic stress began to forage more rapidly (Figure S2).

3.2 | Intensity of foraging

The pattern of intensity of foraging across treatments overall matches the pattern observed for the delay before foraging: foraging was lower when the duration or intensity of the heat stress increased (Figure 2; Table 2). Short heat stresses did not have an effect on foraging intensity (Table 2). Intermediate-duration heat stresses affected the intensity of foraging, and workers exposed to the highest temperature (40°C) showed a lower activity compared to those exposed to 25°C and 30°C (Table 2). Workers exposed to 35°C exhibited an intermediate response, marginally different from the 40°C group ($p = 0.051$). Lastly, workers exposed to chronic heat stress foraged less than those kept under control conditions (Table 2). No significant variation in foraging intensity was observed when comparing different durations (short, intermediate and chronic exposures) within each temperature (all $p > 0.05$) (Figure S3).

3.3 | Mortality of workers and queens

Intense or prolonged heat stresses induced mortality. Workers exposed to a short or intermediate-duration heat shock on the thermal plate experienced some mortality, but only under the highest temperature treatment (40°C), and this mortality was significant only for the intermediate duration. The percentage of colonies in which at least one worker died from heat stress was

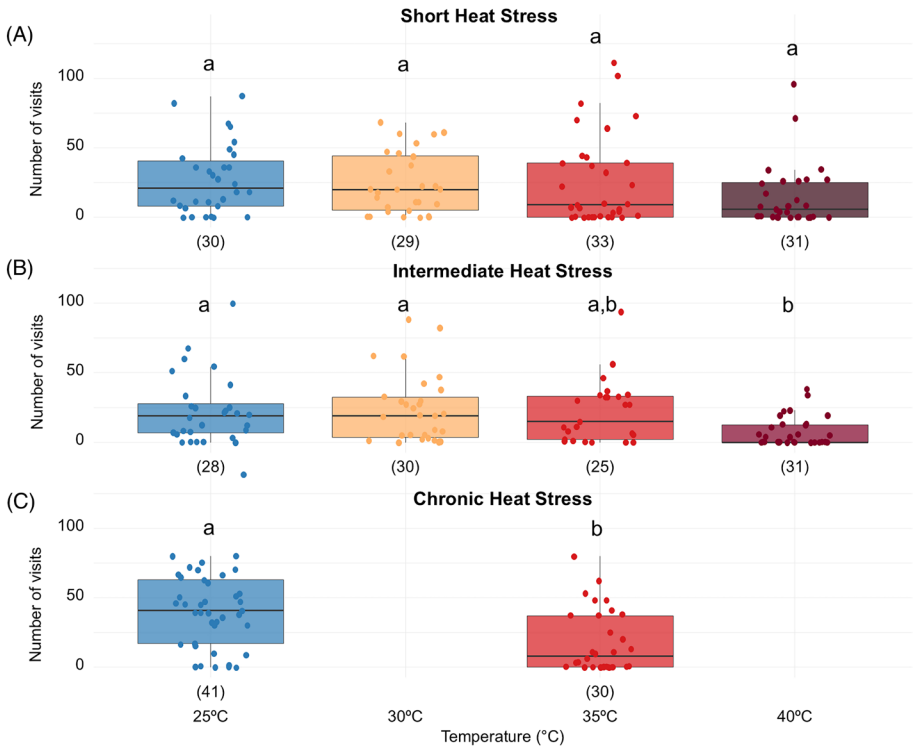

FIGURE 2 Number of foraging visits at different temperatures for each duration of exposure: Short (A), intermediate (B), and chronic (C) heat stress. The box plots show the median, quartiles, and range, with the dots representing the total number of visits per colony. Different letters indicate groups that differ statistically from each other ($p < 0.05$) within a panel. The numbers of colonies tested are given in parentheses.

TABLE 2 GLMM results and estimated marginal means (*emmeans*) for the intensity of foraging depending on the duration of exposure.

(A) GLMM intensity of foraging—short-term exposure					
	Estimate	**SE**	***z* value**	***p*-value**	**Significance**
Intercept	3.326	0.331	10.049	<0.001	***
30°C	−0.238	0.4	−0.594	0.552	n.s.
35°C	−0.41	0.459	−0.894	0.371	n.s.
40°C	−0.916	0.514	−1.782	0.075	n.s.
(B) GLMM intensity of foraging—intermediate exposure					
	Estimate	**SE**	***z* value**	***p*-value**	**Significance**
Intercept	3.091	0.314	9.839	<0.001	***
30°C	0.017	0.392	0.045	0.964	n.s.
35°C	−0.026	0.43	−0.061	0.951	n.s.
40°C	−1.214	0.446	−2.718	0.006	**
(C) *emmeans* intensity of foraging—intermediate exposure					
	Estimate	**SE**	***t* ratio**	***p*-value**	**Significance**
25°C vs. 30°C	−0.017	0.392	−0.045	1	n.s.
25°C vs. 35°C	0.026	0.431	0.061	0.999	n.s.
25°C vs. 40°C	1.214	0.447	2.718	0.037	*
30°C vs. 35°C	0.044	0.397	0.111	0.999	n.s.
30°C vs. 40°C	1.231	0.435	2.835	0.027	*
35°C vs. 40°C	1.187	0.457	2.6	0.051	n.s.
(D) GLMM intensity of foraging—chronic exposure					
	Estimate	**SE**	***z* value**	***p*-value**	**Significance**
Intercept	3.735	0.213	17.536	<0.001	***
35°C:30°C	−0.83	0.329	−2.521	0.011	*

Note: Asterisks indicate statistically significant differences: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

significantly higher for treatment at 40°C than for other treatments, both for short durations (25°C vs. 40°C: $p = 0.001$; 30°C vs. 40°C: $p = 0.001$; 35°C vs. 40°C: $p = 0.001$, Figure 3A) and intermediate durations (25°C vs. 40°C: $p = 0.001$; 30°C vs. 40°C: $p = 0.001$; 35°C vs. 40°C: $p = 0.001$, Figure 3B). We then tested whether the detected worker mortality was significant by comparing the number of workers alive at the beginning and at the end of the heat shock treatment. Worker mortality was not significant for a short duration (Table 3; Figure 3C), while it was for an intermediate duration (Table 3; Figure 3D).

Chronic heat stress also affected worker survival, as well as the queen, since the entire colony was exposed to heat during this treatment (Table 4; Figure 4). Specifically, although the number of workers did not change before and after the experiment in colonies maintained under control conditions (Table 4; Figure 4A), it decreased significantly in colonies maintained under heat stress (Table 4; Figure 4B). Queen mortality was similarly affected. None of the colonies maintained under control conditions (25°C:20°C) lost their queen during the 2 week period, while approximately 30% of the colonies under chronic heat stress (35°C:30°C) did ($p < 0.001$; Figure 4C). Furthermore, five entire colonies initially established at 35°C:30°C did not survive the 2-week treatment. Furthermore, we continued to monitor 20 colonies belonging to the chronic control group at 25°C:20°C and 17 colonies belonging to the chronic stressed group at 35°C:30°C passed the 2-week chronic exposure to track queen mortality over a period of approximately 2 months. None of the queens died in the control group, while all of the queens in the stressed group progressively died, the last queen dying 71 days after the start of experimental treatment (Figure 4D). This shows that heat stress (35°C), which has no effect when it is short or of intermediate duration (Figure 3), can have a strong effect when it is chronic (Figure 4).

4 | DISCUSSION

Our experiments show that heat stress (i.e., heat intensity and duration of exposure to heat) affects two fundamental aspects of colony viability in incipient colonies of *L. niger*, namely, foraging behaviour and survival. Our results aligned with our predictions, as we observed that short-term exposure to high heat was less detrimental than prolonged exposure to more moderate heat. This emphasizes that both the magnitude and duration of heat exposure are critical

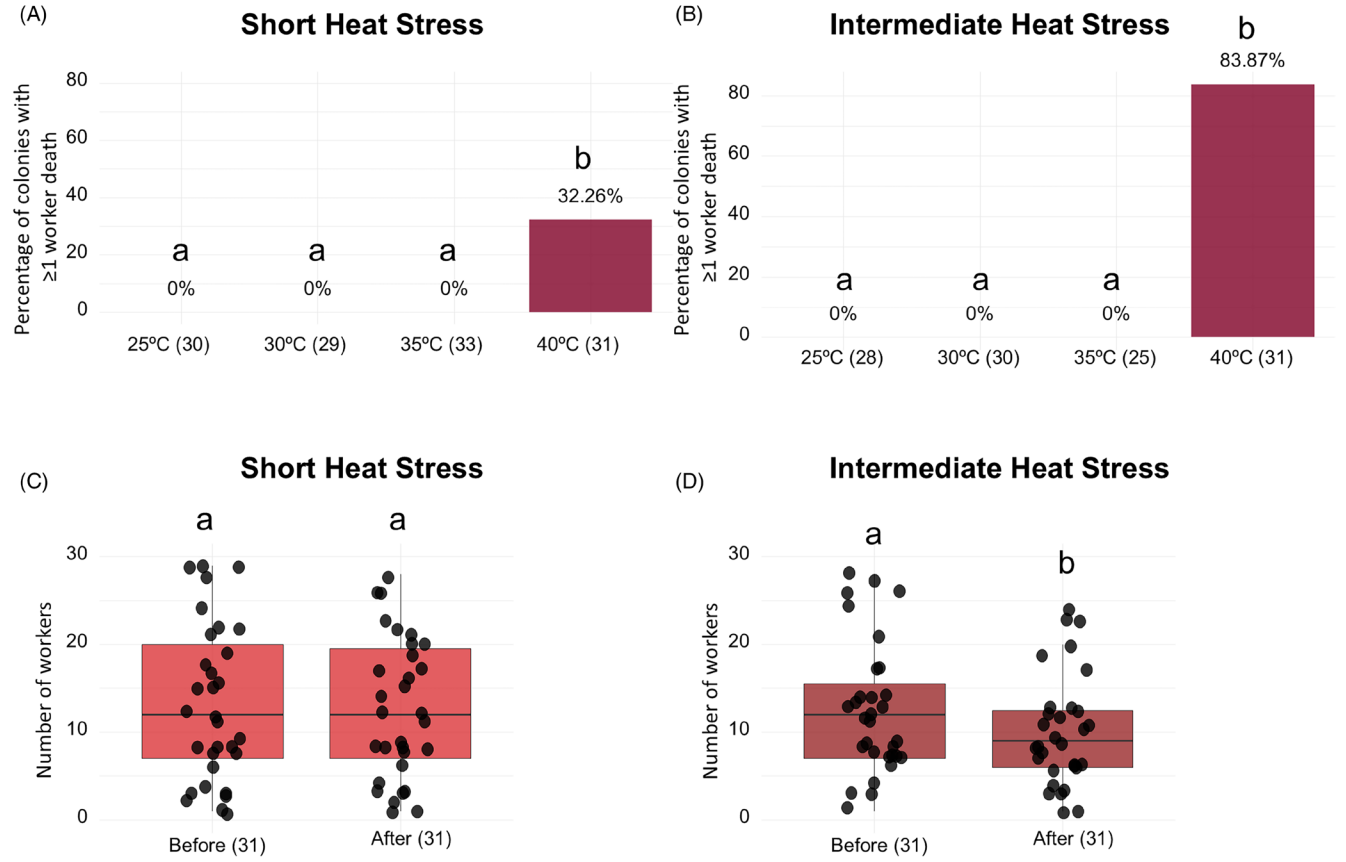

FIGURE 3 Worker mortality after short- and intermediate-duration heat stress at 40°C. The plots show the percentage of colonies where at least one worker died after thermal plate exposure after short (A) and intermediate (B) exposure and the number of workers before and after heat stress for short (C) and intermediate (D) durations. The box plots show the median, quartiles, and range, with dots representing the number of workers per colony. Different letters indicate groups that differ statistically from each other ($p < 0.05$). The numbers of colonies tested are given in parentheses.

TABLE 3 GLMM results for worker mortality registered before and after exposure to thermal plates, depending on the duration of exposure at 40°C.

	Estimate	SE	*z* value	*p*-value	Significance
(A) Short-term exposure to GLMM mortality short-term exposure—40°C					
Intercept	2.401	0.238	10.058	<0.001	***
After thermal plate	−0.049	0.07	−0.706	0.48	n.s.
(B) GLMM mortality intermediate exposure—40°C					
Intercept	2.356	0.124	18.893	<0.001	***
After thermal plate	−0.214	0.075	−2.822	0.004	**

Note: Asterisks indicate statistically significant differences: $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 4 GLMM results for worker mortality registered before and after chronic exposure.

	Estimate	SE	*z* value	*p*-value	Significance
(A) Chronic exposure of GLMM mortality chronic exposure 25°C:20°C					
Intercept	2.589	0.089	28.827	<0.001	***
After 2 weeks	0.042	0.068	0.617	0.537	n.s.
(B) GLMM mortality chronic exposure 35°C:30°C					
Intercept	2.247	0.185	12.122	<0.001	***
After 2 weeks	−0.354	0.112	−3.153	0.001	**

Note: Asterisks indicate statistically significant differences: $^{**}p < 0.01$, $^{***}p < 0.001$.

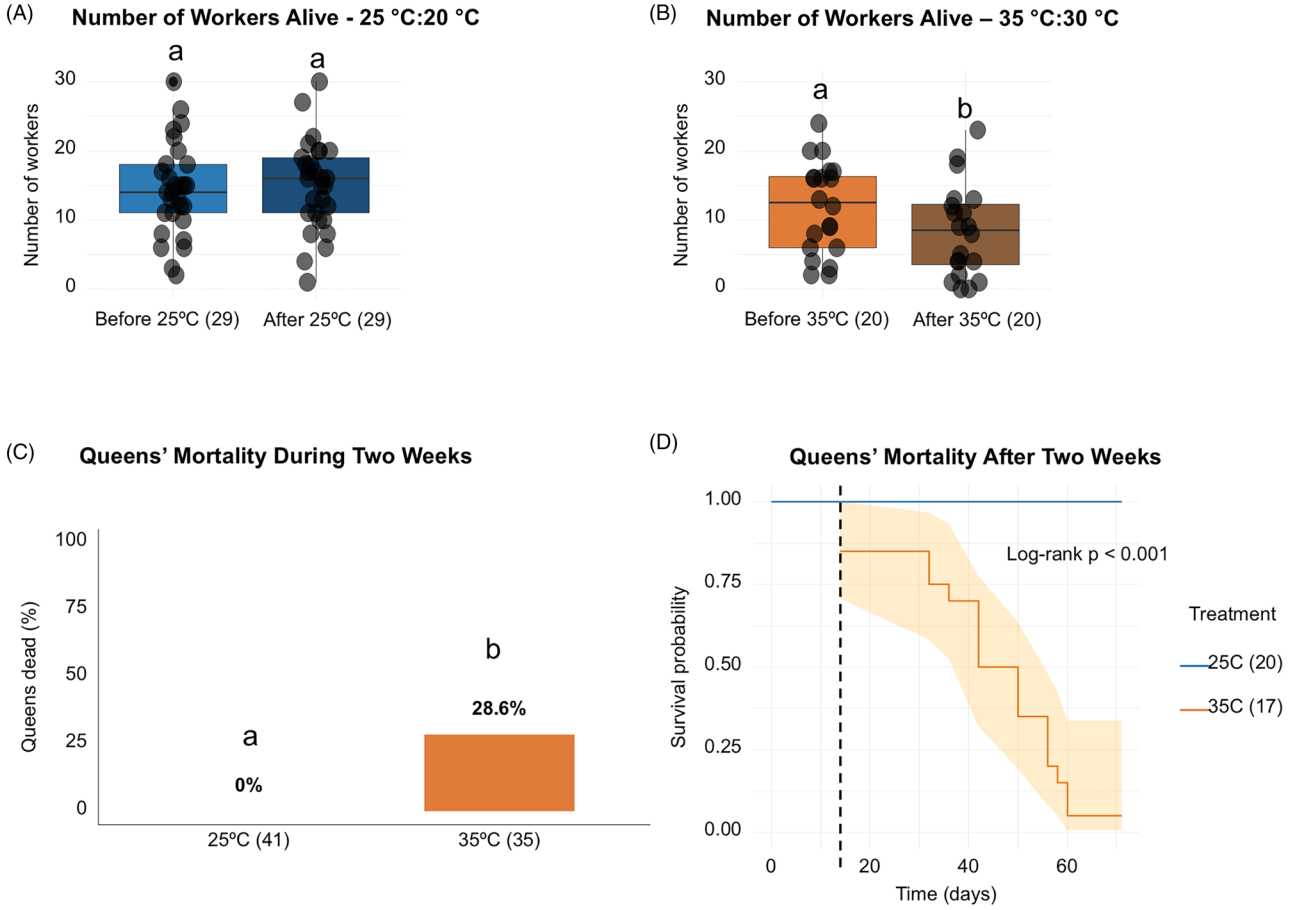

FIGURE 4 Worker and queen mortality after chronic treatment. The graphs show the number of workers in the colonies before and after exposure to chronic treatment for a subset of 29 colonies under control conditions (25°C:20°C, A) and 20 heat stressed colonies (35°C:30°C, B), the percentage of colonies where the queen was dead before the foraging experiment was performed (all colonies) (C), and the mortality of the queens after their colonies were subjected to 2 weeks of exposure plus the foraging experiment for a subset of colonies (D) ($n = 20$ for 25°C:20°C and $n = 17$ for 35°C:30°C). The dashed line represents the end of chronic exposure when the colonies were tested for foraging. The queen's mortality was then monitored until the last queen of each treatment had died. The box plots show the median, quartiles, and range, with dots representing the number of workers per colony. Different letters indicate groups that differ statistically from each other ($p < 0.05$). The numbers of colonies tested are given in parentheses.

in determining colony thermal vulnerability, in agreement with the TDT framework.

Incipient colonies may be more susceptible to heat stress than larger colonies, because incipient colonies have nanitic workers (Madsen & Offenberg, 2017) and that small workers typically have lower heat resistance than larger conspecific workers in ants (Arnan et al., 2022; Nascimento et al., 2022). In the study species, this aspect has not yet been confirmed by direct comparison of heat resistance between nanitic and normal workers. Incipient colonies are also likely to be more susceptible to heat because they have very few workers. This makes each worker lost to heat a major loss compared to more populous colonies, and also makes adjustment to heat, such as excavating a deeper nest (García Ibarra et al., 2024), more difficult. Note that queens and incipient colonies of *L. niger* have evolved strategies that shorten the duration of the incipient colony stage in response to the strong intraspecific competition (Sommer & Hölldobler, 1995; Aron et al., 2009), and this shortening may help in resisting heat stress. Queens often found new colonies by pleometrosis, where several unrelated queens jointly found a colony, which results in faster colony growth, before monogyny is restored (Sommer & Hölldobler, 1995; Aron et al., 2009). Additionally, incipient colonies engage in brood raiding. Foragers enter and steal pupae from other incipient colonies, which results in the raiding colony very rapidly increasing its workforce (Madsen & Offenberg, 2017). Despite the above evidence that incipient colonies are particularly susceptible to heat, a direct comparison between the heat sensitivity of incipient and mature *L. niger* colonies remains to be made. Interestingly, in approximately 50% of colonies under chronic heat stress treatment, at least one worker still visited the foraging area even after 2 weeks of exposure. This suggests that a certain degree of resilience may occur under prolonged thermal stress in specific cases. *L. niger* colonies are synanthropic (adapted to live near humans) and exhibit high plasticity (Czechowski, 2024; Konorov et al., 2017), and we hypothesize that this trait may contribute to its high prevalence in urban environments.

Previous studies have shown that colonies inhabiting warmer environments can exhibit higher heat tolerance than those from cooler

environments (e.g., *Temnothorax curvispinosus*; Martin et al., 2019), suggesting that such differences may be shaped by genetic differentiation between populations. This capacity to cope with temperature variation has also been observed in the leaf-cutting ant *Atta sexdens rubropilosa*, where urban-derived workers survived longer under thermal stress than those from non-urban colonies (Angilletta Jr et al., 2007), although the underlying mechanisms (plasticity and/or genetic differentiation) remain unresolved. Future studies may test whether urban and rural *L. niger* colonies differ in heat resistance and underlying mechanisms (genetic versus phenotypic plasticity).

Similar patterns have been reported in other insect systems, where the rearing temperature or prior thermal exposure can differentially affect individuals depending on the intensity of that exposure. For example, in *B. terrestris*, elevated rearing temperatures culminate in a drastic reduction in both the number of foragers and the total foraging trips (Gérard et al., 2022). Colonies reared at 33°C showed approximately 93% fewer foraging trips per worker and 79% fewer trips in total compared to those reared at 27°C. These results were partly attributed to colony size, as colonies reared at 33°C produced approximately 40% fewer workers (Gérard et al., 2022). In our study, the reduced foraging activity observed under medium-term exposure at 40°C and long-term exposure at 35°C may reflect a similar pattern, with increased mortality resulting in fewer workers available to sustain foraging. Furthermore, Gérard et al. (2024) found that the temperature during the foraging itself can shape the performance of bumblebees, with those foraging at 32°C showing higher visitation rates compared to those foraging at 24°C. Although our study did not examine the impact of temperature during foraging itself, previous work has shown that foraging dynamics in *L. niger* is temperature dependent. Specifically, an increase of 3°C relative to the mean summer temperature at the study site (20°C) improved foraging performance, while an increase of 6°C disrupted it (Blanchard et al., 2021). Due to our approach, we were unable to calculate the per capita foraging efficiency and instead assessed the colony-level foraging output. Future studies using a similar design, but tracking individual workers, may help unravel the effects of temperature and exposure duration on foraging behaviour in *L. niger*, both on individual and collective scales.

Temperature can constrain ants in various ways, including their metabolism (Shik et al., 2019), immune responses (da Silva & Monnin, 2026), individual and nest development (García Ibarra et al., 2024; Porter, 1988; Roux et al., 2026) and behavioural performance (Blanchard et al., 2021; Hurlbert et al., 2008; Trigos-Peral et al., 2024). Therefore, in our work, the reduced performance observed as colonies experienced increased temperatures and prolonged exposure durations may reflect underlying physiological stress that compromised behavioural functioning and survival. Insects typically show higher metabolic rates under increasing temperatures (Gérard et al., 2024), which, beyond an optimal thermal range, can lead to physiological stress, including oxidative damage and energy depletion. This burden likely compromises behaviour, especially under prolonged exposure, as seen in our intermediate treatments (40°C) and chronic treatments (35°C:30°C). Increased metabolic rates associated with high temperatures elevate oxygen consumption in ectotherms, which in turn enhances the production of reactive oxygen species (ROS) as metabolic by-products (Finkel & Holbrook, 2000; Rollins-Smith & Le Sage, 2023). Based on the link between high temperature exposure, elevated metabolic rates, increased ROS production, and reduced lifespan, we hypothesize that the high mortality observed among workers and queens reared under chronic stress of 35°C:30°C stem from these cascading effects. This is particularly plausible given that chronic stress affects heat-induced damage repair, as proposed in the TLS framework (Arnold et al., 2025).

However, this mechanism only partially explains the performance observed in colonies exposed to the highest temperatures, particularly those in which no foraging visits were recorded or only a few. On the contrary, some colonies, even under the most suboptimal conditions, managed to forage, although with longer latencies compared to colonies from other treatments. In *B. terrestris*, colonies that underwent a reduction in the workforce after a thermal challenge demonstrated behavioural resilience by increasing the number of workers available to engage in foraging activities (Gérard et al., 2023). In our study, this compensatory strategy may not have been feasible due to the overall low number of workers resulting from increased mortality and the incipient stage of the colonies. Under these circumstances, a plausible alternative strategy may involve the concentration of foraging activity in one or a few available workers operating beyond their typical foraging workload. These results suggest that a return to normal performance may require additional time in incipient colonies of *L. niger* under suboptimal conditions.

Beyond impacts related to foraging, increasing soil surface temperatures may also affect nest establishment and the development of incipient colonies. For example, young *L. niger* colonies with a few hundred workers respond to increased surface temperatures by excavating deeper nests (García Ibarra et al., 2024). Such behavioural responses likely involve energetic and temporal costs, which may be particularly relevant for incipient colonies with a very limited workforce. The combination of a reduced number of workers and nanitic workers can determine how incipient colonies cope with thermal stress. A comprehensive understanding of the underlying effects of heat stress on incipient ant colonies is critical in the context of global climate change, where the increasing frequency and duration of episodes of extreme heat events could affect colony establishment and species persistence.

Future studies should track individual activity and mortality over time, ideally in controlled systems that enable fine-scale behavioural quantification. Long-term monitoring of colony development in artificial 2D nests may offer a more realistic and detailed view of how thermal stress shapes the founding phase, colony ontogeny, task allocation, and resilience. Complementary experimental designs incorporating parallel assays of incipient and mature colonies under comparable thermal regimes would be particularly valuable to unravel the effects of worker size and colony size on thermal resilience in social insects. For instance, depending on whether CT_{max} likely depends on worker size, colonies with more resources may invest in the

production of larger workers. Although the TDT/TLS framework was not explicitly applied here, future studies could use it to assess how parameters such as CT_{max}, thermal breadth (a proxy of thermal tolerance, since it considers both CT_{max} and CT_{min}), and damage repairs vary between castes, age, and stages of development of the colony in ants.

AUTHOR CONTRIBUTIONS

Rafael Carvalho da Silva and Thibaud Monnin conceived the ideas, designed the methodology, and led the writing of the manuscript. Rafael Carvalho da Silva, Maëlys Prost, and Angélique Bultelle carried out the experiments. Rafael Carvalho da Silva analysed the data. All authors contributed to the manuscript preparation and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank the associate editor and the three reviewers for their valuable comments and suggestions in the earlier versions of our manuscript. RCS thanks Sorbonne Université' SOUND programme for sponsoring his postdoctoral project (« *Avec le soutien de l'Alliance Sorbonne Université dans le cadre du projet « SOUND—pour un nouvel engagement » financé par l'ANR au titre de France 2030 (ANR-22-EXES-0004)* »). We would like to thank Viviana Di Pietro, Anneline Mattens, An Vandoren, and Helena Mendes Ferreira for collecting the mated queens that were used in our experiments.

FUNDING INFORMATION

This work was supported by the Sorbonne University Alliance through the "SOUND – pour un nouvel engagement" project, funded by the French National Research Agency (ANR) under the France 2030 programme (grant no. ANR-22-EXES-0004).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the FigShare Repository https://doi.org/10.5061/dryad.vdncjsz9x (da Silva et al., 2026).

STATEMENT OF INCLUSION

Our study includes one researcher from South America and three researchers from the country where the study was developed.

ORCID

Rafael Carvalho da Silva https://orcid.org/0000-0003-2674-8976

REFERENCES

Andrew, N. R., Hart, R. A., Jung, M. P., Hemmings, Z., & Terblanche, J. S. (2013). Can temperate insects take the heat? A case study of the physiological and behavioural responses in a common ant, *Iridomyrmex purpureus* (Formicidae), with potential climate change. *Journal of Insect Physiology*, *59*(9), 870–880. https://doi.org/10.1016/j.jinsphys.2013.06.003

Angilletta, M. J., Jr. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press.

Angilletta, M. J., Jr., Wilson, R. S., Niehaus, A. C., Sears, M. W., Navas, C. A., & Ribeiro, P. L. (2007). Urban physiology: City ants possess high heat tolerance. *PLoS One*, *2*(2), e258. https://doi.org/10.1371/journal.pone.0000258

Arnan, X., Lázaro-González, A., Beltran, N., Rodrigo, A., & Pol, R. (2022). Thermal physiology, foraging pattern, and worker body size interact to influence coexistence in sympatric polymorphic harvester ants (*Messor* spp.). *Behavioral Ecology and Sociobiology*, *76*(6), 80. https://doi.org/10.1007/s00265-022-03186-6

Arnold, P. A., Noble, D. W., Nicotra, A. B., Kearney, M. R., Rezende, E. L., Andrew, S. C., Briceño, V. F., Buckley, L. B., Christian, K. A., Clusella-Trullas, S., Geange, S. R., Guja, L. K., Robles, O. J., Kefford, B. J., Kellermann, V., Leigh, A., Marchin, R. M., Mokany, K., & Bennett, J. M. (2025). A framework for modelling thermal load sensitivity across life. *Global Change Biology*, *31*, e70315. https://doi.org/10.1111/gcb.70315

Aron, S., Steinhauer, N., & Fournier D. (2009). Influence of queen phenotype, investment and maternity apportionment on the outcome of fights in cooperative foundations of the ant Lasius niger. *Animal Behaviour*, *77*(5), 1067–1074. https://doi.org/10.1016/j.anbehav.2009.01.009

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*, 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Blanchard, S., Van Offelen, J., Verheggen, F., & Detrain, C. (2021). Towards more intimacy: Moderate elevation of temperature drives increases in foraging and mutualistic interactions between *Lasius niger* and *Aphis fabae*. *Ecological Entomology*, *46*(2), 406–418. https://doi.org/10.1111/een.12982

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9/2*, 378–400.

Canal Domenech, B., & Fricke, C. (2022). Recovery from heat-induced infertility—A study of reproductive tissue responses and fitness consequences in male *Drosophila melanogaster*. *Ecology and Evolution*, *12*(12), e9563. https://doi.org/10.1002/ece3.9563

Carroll, C. R., & Janzen, D. H. (1973). Ecology of foraging by ants. *Annual Review of Ecology and Systematics*, *4*, 231–257. https://doi.org/10.1146/annurev.es.04.110173.001311

Cerdá, X., Retana, J., & Cros, S. (1998). Critical thermal limits in Mediterranean ant species: Trade-off between mortality risk and foraging performance. *Functional Ecology*, *12*(1), 45–55. https://doi.org/10.1046/j.1365-2435.1998.00160.x

Corley, R. B., Dawson, W., & Bishop, T. R. (2023). A simple method to account for thermal boundary layers during the estimation of CT_{max} in small ectotherms. *Journal of Thermal Biology*, *116*, 103673. https://doi.org/10.1016/j.jtherbio.2023.103673

Czechowski, W. (2024). Where is the nest? A peculiar mode of synanthropic nesting of *Lasius niger* (L.) (Hymenoptera: Formicidae). *Annals of the Upper Silesian Museum in Bytom, Entomology*, *33*(9), 1–16. https://doi.org/10.5281/zenodo.14546367

Czechowski, W., Radchenko, A., Czechowska, W., & Vepsäläinen, K. (2012). *The ants of Poland with reference to the myrmecofauna of Europe*. Natura Optima dux Foundation.

da Silva, R. C., & Monnin, T. (2026). Thermal stress impairs survival and immune responses in ant founding queens. *Biology Letters*, *22*(6), 20260072. https://doi.org/10.1098/rsbl.2026.0072

da Silva, R. C., Pros, M., Bultelle, A., & Monnin, T. (2026). Data from: Effects of heat stress on the foraging activity of incipient ant colonies. *FigShare Repository*. https://doi.org/10.6084/m9.figshare.32731122

de Souza, H. J., & Delabie, J. H. C. (2021). Ecosystem engineers: Ants and termites. In C. K. Starr (Ed.), *Encyclopedia of social insects*. Springer. https://doi.org/10.1007/978-3-030-28102-1_186

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*(6809), 239–247. https://doi.org/10.1038/35041687

García Ibarra, F., Jouquet, P., Bottinelli, N., Bultelle, A., & Monnin, T. (2024). Experimental evidence that increased surface temperature affects bioturbation by ants. *Journal of Animal Ecology*, *93*(3), 319–332. https://doi.org/10.1111/1365-2656.14040

Gérard, M., Cariou, B., Henrion, M., Descamps, C., & Baird, E. (2022). Exposure to elevated temperature during development affects bumblebee foraging behavior. *Behavioral Ecology*, *33*(4), 816–824. https://doi.org/10.1093/beheco/arac045

Gérard, M., Gardelin, E., Lehmann, P., Roberts, K. T., Sepúlveda-Rodríguez, G., Sisquella, C., & Baird, E. (2024). Experimental elevated temperature affects bumblebee foraging and flight speed. *Proceedings. Biological sciences*, *291*(2033), 20241598. https://doi.org/10.1098/rspb.2024.1598

Gérard, M., Magitteri, A., Sisquella, C., Gardelin, E., Tesse, J., & Baird, E. (2025). Elevated temperatures shape cognitive performance and foraging preferences in free-flying bumblebees. *Royal Society Open Science*, *12*(12), 251306. https://doi.org/10.1098/rsos.251306

Gérard, M., Marchand, J., Zanutto, J., & Baird, E. (2023). Resilience of bumblebee foraging behavior despite colony size reduction. *Frontiers in Insect Science*, *2*, 1073380. https://doi.org/10.3389/finsc.2022.1073380

Guiraud, M., Cariou, B., Henrion, M., Baird, E., & Gérard, M. (2021). Higher developmental temperature increases queen production and decreases worker body size in the bumblebee *Bombus terrestris*. *Journal of Hymenoptera Research*, *88*, 39–49. https://doi.org/10.3897/jhr.88.73532

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Springer-Verlag.

Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, *4*(5), 131–135. https://doi.org/10.1016/0169-5347(89)90211-5

Hurlbert, A. H., Ballantyne, F., & Powell, S. (2008). Shaking a leg and hot to trot: The effects of body size and temperature on running speed in ants. *Ecological Entomology*, *33*(1), 144–154. https://doi.org/10.1111/j.1365-2311.2007.00962.x

Jørgensen, L. B., Malte, H., & Overgaard, J. (2019). How to assess drosophila heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits. *Functional Ecology*, *33*, 629–642. https://doi.org/10.1111/1365-2435.13279

Kassambara, A. (2018). *ggpubr: 'ggplot2' based publication ready plots*. R package version, 2.

Kassambara, A., Kosinski, M., & Biecek, P. (2016). *survminer: Drawing Survival Curves using 'ggplot2'*. CRAN: Contributed Packages.

Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, *27*(6), 1415–1423. https://doi.org/10.1111/1365-2435.12145

Konorov, E. A., Nikitin, M. A., Mikhailov, K. V., Lysenkov, S. N., Belenky, M., Chang, P. L., Nuzhdin, S. V., & Scobeyeva, V. A. (2017). Genomic exaptation enables *Lasius niger* adaptation to urban environments. *BMC Evolutionary Biology*, *17*, 39. https://doi.org/10.1186/s12862-016-0867-x

Leong, C. M., Hui, T. Y., & Guénard, B. (2023). The role of body mass in limiting post heat-coma recovery ability in terrestrial ectotherms. *Ecology and Evolution*, *13*(6), e10218. https://doi.org/10.1002/ece3.10218

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(44), E10397–E10406. https://doi.org/10.1073/pnas.1722477115

Madsen, N. E., & Offenberg, J. (2017). Effect of pleometrosis and brood transplantation on colony growth of the black garden ant, *Lasius niger*. *Asian Myrmecology*, *9*, e009003. https://doi.org/10.20362/am.009003

Martin, R. A., Chick, L. D., Yilmaz, A. R., & Diamond, S. E. (2019). Evolution, not transgenerational plasticity, explains the adaptive divergence of acorn ant thermal tolerance across an urban–rural temperature cline. *Evolutionary Applications*, *12*(8), 1678–1687. https://doi.org/10.1111/eva.12826

Menzel, F., & Feldmeyer, B. (2021). How does climate change affect social insects? *Current Opinion in Insect Science*, *46*, 10–15. https://doi.org/10.1016/j.cois.2021.01.005

Moradinour, Z., Wiklund, C., Miettinen, A., Gérard, M., & Baird, E. (2023). Exposure to elevated temperature during development affects eclosion and morphology in the temperate *Pieris napi* butterfly (Lepidoptera: Pieridae). *Journal of Thermal Biology*, *118*, 103721. https://doi.org/10.1016/j.jtherbio.2023.103721

Nascimento, G., Câmara, T., & Arnan, X. (2022). Critical thermal limits in ants and their implications under climate change. *Biological Reviews*, *97*(4), 1287–1305. https://doi.org/10.1111/brv.12843

Parr, C. L., & Bishop, T. R. (2022). The response of ants to climate change. *Global Change Biology*, *28*(10), 3188–3205. https://doi.org/10.1111/gcb.16140

Peeters, C., & Ito, F. (2001). Colony dispersal and the evolution of queen morphology in social hymenoptera. *Annual Review of Entomology*, *46*(1), 601–630. https://doi.org/10.1146/annurev.ento.46.1.601

Peralta-Maraver, I., & Rezende, E. L. (2021). Heat tolerance in ectotherms scales predictably with body size. *Nature Climate Change*, *11*(1), 58–63. https://doi.org/10.1038/s41558-020-00938-y

Porter, S. D. (1988). Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. *Journal of Insect Physiology*, *34*(12), 1127–1133. https://doi.org/10.1016/0022-1910(88)90215-6

Porter, S. D., & Tschinkel, W. R. (1986). Adaptive value of nanitic workers in newly founded red imported fire ant colonies (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, *79*(4), 723–726. https://doi.org/10.1093/aesa/79.4.723

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology*, *28*, 799–809. https://doi.org/10.1111/1365-2435.12268

Roeder, D. V., Paraskevopoulos, A. W., & Roeder, K. A. (2022). Thermal tolerance regulates foraging behaviour of ants. *Ecological Entomology*, *47*(3), 331–338. https://doi.org/10.1111/een.13118

Roeder, K. A., Roeder, D. V., & Bujan, J. (2021). Ant thermal tolerance: A review of methods, hypotheses, and sources of variation. *Annals of the Entomological Society of America*, *114*(4), 459–469. https://doi.org/10.1093/aesa/saab018

Rollins-Smith, L. A., & Le Sage, E. H. (2023). Heat stress and amphibian immunity in a time of climate change. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *378*(1882), 20220132. https://doi.org/10.1098/rstb.2022.0132

Roux, A., Zimmermann, A., Goubault, M., Villalta, I., & Libbrecht, R. (2026). Impact of heat stress on colony foundation in ants. *Biology Letters*, *22*, 20260016. https://doi.org/10.1098/rsbl.2026.0016

Sankovitz, M., & Purcell, J. (2021). Ant nest architecture is shaped by local adaptation and plastic response to temperature. *Scientific Reports*, *11*(1), 1–10. https://doi.org/10.1038/s41598-021-02491-w

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Shik, J. Z., Arnan, X., Oms, C. S., Cerdá, X., & Boulay, R. (2019). Evidence for locally adaptive metabolic rates among ant populations along an

elevational gradient. *Journal of Animal Ecology*, *88*(8), 1240–1249. https://doi.org/10.1111/1365-2656.13007

Sommer, K., & Hölldobler, B. (1995). Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. *Animal Behaviour*, *50*(2), 287–294. https://doi.org/10.1006/anbe.1995.0244

Spicer, M. E., Stark, A. Y., Adams, B. J., Kneale, R., Kaspari, M., & Yanoviak, S. P. (2017). Thermal constraints on foraging of tropical canopy ants. *Oecologia*, *183*(4), 1007–1017. https://doi.org/10.1007/s00442-017-3825-4

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(15), 5610–5615. https://doi.org/10.1073/pnas.1316145111

Therneau, T. (2015). A package for survival analysis in S. *R Package Version*, *2*(7), 2014.

Trigos-Peral, G., Maák, I. E., Schmid, S., Chudzik, P., Czaczkes, T. J., Witek, M., Casacci, L. P., Sánchez-García, D., Lőrincz, Á., Kochanowski, M., & Heinze, J. (2024). Urban abiotic stressors drive changes in the foraging activity and colony growth of the black garden ant *Lasius niger*. *Science of the Total Environment*, *915*, 170157. https://doi.org/10.1016/j.scitotenv.2024.170157

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*, 571–573. https://doi.org/10.1126/science.aaa4984

Vollet-Neto, A., Menezes, C., & Imperatriz-Fonseca, V. L. (2015). Behavioural and developmental responses of a stingless bee (*Scaptotrigona depilis*) to nest overheating. *Apidologie*, *46*, 455–464. https://doi.org/10.1007/s13592-014-0338-6

Wickham, H. (2016). Data analysis. In *ggplot2: Elegant graphics for data analysis* (pp. 189–201). Springer International Publishing.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Summary of colonies used at least twice in the foraging assays.

Figure S1: Colony size at the onset of the experiment. Plots show the number of workers and larvae for colonies used for short (10 min, panels A and B), intermediate (1 h, panels C and D) and chronic exposures (2 weeks, panels E and F). Boxplots show median, quartiles, and range with dots representing the total number of workers (A, C, and E) or larvae (B, D and F) per colony. Different letters indicate the groups that differ statistically from each other ($p < 0.05$).

Figure S2: Delay before the onset of foraging according to the different duration exposure for each temperature. Survival curves represent the cumulative proportion of colonies for which foraging started over time for 25°C (A), 30°C (B), 35°C (C) and 40°C (D) exposed colonies. A steeper curve indicates a shorter delay, while flatter curves reflect a slower or less consistent response.

Figure S3: Number of foraging visits according to the different duration exposure for each temperature (A) 25°C, (B) 30°C, (C) 35°C and (D) 40°C. Boxplots show median, quartiles, and range, with dots showing representing the total number of visits per colonies. No differences were detected among the groups.

How to cite this article: da Silva, R. C., Prost, M., Bultelle, A., & Monnin, T. (2026). Effects of heat stress on the foraging activity of incipient ant colonies. *Journal of Animal Ecology*, *00*, 1–13. https://doi.org/10.1111/1365-2656.70337