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Urban abiotic stressors drive changes in the foraging activity and colony growth of the black garden ant *Lasius niger*

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1 **Urban abiotic stressors drive changes in the foraging activity and colony growth of the**
 2 **black garden ant *Lasius niger***

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12 **Highlights**

- 13 ● Ant foraging activity was generally higher in urban areas.
- 14 ● In the smaller and cooler cities, ant activity was higher in rural areas.
- 15 ● Results of a laboratory study followed the same trend found in the field study.
- 16 ● Urban locomotory traits suggested adaptations to weaker competitive pressures.
- 17 ● In urban areas, brood developed faster but worker mortality increased.

18 **Abstract**

19 Changes in habitat characteristics are known to have profound effects on biotic communities
 20 and their functional traits. In the context of an urban-rural gradient, urbanisation drastically
 21 alters abiotic characteristics, e.g., by increasing environmental temperatures and through light
 22 pollution. These abiotic changes significantly impact the functional traits of organisms,

23 particularly insects. Furthermore, changes in habitat characteristics also drive changes in the
24 behavioural traits of animals, allowing them to adapt and thrive in new environments. In our
25 study, we focused on the synanthropic ant species *Lasius niger* as a model organism. We
26 conducted nocturnal field observations and complemented them with laboratory experiments to
27 investigate the influence of night warming (NW) associated with Urban Heat Islands (UHI),
28 light pollution (ALAN), and habitat type on ant foraging behaviour. In addition, we investigated
29 the influence of elevated temperatures on brood development and worker mortality. Our
30 findings revealed that urban populations of *L. niger* were generally more active during the night
31 compared to their rural counterparts, although the magnitude of this difference varied with
32 specific city characteristics. In laboratory settings, higher temperatures and continuous
33 illumination were associated with increased activity level in ants, again differing between urban
34 and rural populations. Rural ants exhibited more locomotion compared to their urban
35 counterparts when maintained under identical conditions, which might enable them to forage
36 more effectively in a potentially more challenging environment. High temperatures decreased
37 the developmental time of brood from both habitat types and increased worker mortality,
38 although rural colonies were more strongly affected. Overall, our study provides novel insights
39 into the influence of urban environmental stressors on the foraging activity pattern and colony
40 development of ants. Such stressors can be important for the establishment and spread of
41 synanthropic ant species, including invasive ones, and the biotic homogenization of
42 anthropogenic ecosystems.

43 **Keywords:** urbanisation, functional traits, night warming, artificial illumination, larval
44 development, locomotion

45

46 1. Introduction

47 The current Anthropocene Epoch is characterised by the strong influence of human activities
48 on climate and the environment. One of its main characteristics is the rapid spread of
49 urbanisation, resulting in more than 50% of the human population living in urban areas and an
50 estimated 1.8 – 5.9-fold increase of the global total amount of urban land over the century (Gao
51 and O’Neill, 2020). Generally, all types of land use are associated with changes in the abiotic
52 and biotic characteristics of habitats. However, the extent of their impact is not uniform.
53 Urbanisation is considered one of the most drastic habitat changes, as it shapes both the
54 landscapes and the environmental characteristics and therefore threatens native biodiversity
55 (McKinney 2008, Shochat et al. 2010, Rastandeh et al. 2017, de Barros Ruas et al. 2022). The
56 reduction of green areas, the release of heat absorbed by the concrete and buildings during the
57 day, and the interference of buildings with wind flow result in an increase in environmental
58 temperature within cities. The strength of this phenomenon known as urban heat island (UHI)
59 has been linked to the size of the city, the compactness of its structures, and its shape (Zhou et
60 al. 2017) since these factors contribute to the cities’ hermeticity and their capacity to cool down.

61 UHIs are characterised by higher temperatures during the night (night warming – NW). This
62 results in differences between urban and rural populations of organisms, as temperature is
63 typically linked with their functional traits, behaviour and survival (Bennett et al. 2021). For
64 example, the phenology of both plants and animals varies along an urban-rural gradient, with
65 an earlier start of the growing (plants) and changes in the activity (animals) season in urban
66 areas (i.e., Møller et al. 2015, Merckx et al. 2021, Liu et al. 2023). In addition, urban areas are
67 characterised by light pollution (artificial light at night - ALAN), which has already been shown
68 to have consequences on human health and on activity patterns in animals (Buczowski and
69 Richmond 2012, Owens and Lewis 2018, Widmer et al. 2022, Burt et al. 2023). The described
70 responses of animals to ALAN are diverse; for example, ALAN enlarges the activity period of
71 the diurnal feral pigeon (Leveau 2020), reduces the nocturnal song behaviour of willie wagtails

72 (Dickerson et al. 2022), and affects the navigation of turtles to the ocean after hatching as they
73 may be attracted by light along the shore. Moreover, in rodents, ALAN reduces their activity
74 period both during the day and night (Sanders et al. 2021), influencing their anxiety-like
75 behaviour and their food intake at night due to decreased melatonin production connected with
76 the neuroendocrine regulation under light conditions (Keen-Rhinehart and Bartness 2005,
77 Russart & Nelson 2018). Some invertebrate species may be evolving in response to ALAN, for
78 example by becoming less attracted to lights, or less repelled by them (Altermatt and Ebert
79 2016, Czaczkes et al. 2018).

80 Insects are very important components of our ecosystems (Scudder 2017) and ants are
81 considered the most abundant insects on earth (Schultheiss et al. 2022). Together with their
82 quick response to habitat change, this makes ants suitable bioindicators in evolutionary-
83 ecological studies. Several studies have shown how urbanisation shapes their communities (i.e.,
84 Carpintero and Reyes-López 2014, Guénard et al. 2015, Brassard et al. 2021), however, recent
85 studies focus mostly on the physiological and behavioural mechanisms associated with these
86 changes and their underlying causes, including temperature and illumination. For example,
87 workers of the acorn ant *Temnothorax curvispinosus* from urban populations have been shown
88 to cope with UHI by modifying their metabolic rates and increasing their thermal resistance
89 (Martin et al. 2019, Chick et al. 2020, Diamond and Martin 2020). These studies also suggest
90 the potential heritability of these traits by showing that they are maintained in subsequent
91 generations of workers reared under controlled laboratory conditions.

92 Moreover, temperature also plays a major role in the growth of the colony. As poikilothermic
93 animals, both the activity of individuals and larval development are strongly related to the
94 temperature. Each species shows a range of temperatures that allows the optimal development
95 of the brood, and within that range, an increase in temperatures shortens the development time
96 (Abril et al. 2010) and boosts colony growth (Porter 1988). Notwithstanding, although

97 temperatures below this range stop development, those above the range can also have
98 detrimental consequences due to the mortality of individuals by stress (Penick et al. 2017).
99 Therefore, in urban environments, the faster development of brood might be compensated with
100 the negative impact of mortality by heat stress. However, whether the increase in temperatures
101 overall benefits or harms colony growth or whether the impact of the UHI on colony growth is
102 similar in urban and rural populations is not known yet.

103 Like temperature, urban light pollution also affects the composition of arthropod communities
104 (Davies et al. 2012), their abundance and functional traits, like navigation capabilities (Owens
105 and Lewis 2018). For example, ants use visual, chemical, and geomagnetic cues (Mannino et
106 al. 2023) for orientation. During foraging their reliance on chemical cues varies with ambient
107 light levels (Jones et al. 2019). A reduction of pheromones deposition in the presence of
108 artificial light at night in urban areas might explain the increase of ant activity during nights
109 (Brown et al. 2023).

110 Structurally, urban areas are characterised by the homogenization of their green landscapes and
111 biodiversity. Compared to rural ones, the reduction in the abundance of predators and
112 interspecific competitors might facilitate the search for food, particularly for ants, like *Lasius*
113 *niger*, which rely on trophobiotic interactions with aphids (Korányi et al. 2021). Decreased risks
114 of predation might lead to adaptations in locomotory traits to increase foraging success, as has
115 been found in other scenarios: thermophilic ants run faster and show a more efficient homing
116 when soil temperature increases (Ślipiński and Cerdá 2022). We hypothesise that the more
117 frequent interactions and higher risk of predation during foraging in rural habitats serve as an
118 environmental filter for certain locomotory skills.

119 Our understanding of how urbanisation influences ant activity patterns and locomotion is still
120 limited. For example, although NW and ALAN are considered among the strongest habitat

121 alterations, their effects on insects have rarely been studied together (Tougeron and Sanders
122 2023). Similarly, despite their important effect on the foraging success, whether locomotory
123 traits differ between urban and rural populations has rarely been investigated. Thus, we
124 investigated the effects of urban heat island, night warming, light pollution, and habitat type on
125 the foraging activity and locomotory traits in the widespread synanthropic ant species *Lasius*
126 *niger*. We carried out a combination of field observations and laboratory experiments to test the
127 following hypotheses: 1) NW and ALAN lead to a higher foraging activity and their influence
128 is stronger in urban populations, 2) locomotory traits differ between ant populations with faster
129 and less explorative workers in urban populations than in rural ones, and 3) colony growth
130 might be boosted in urban habitats due to the higher temperatures.

131 **2. Material and methods**

132 **2.1. Study areas**

133 The foraging behaviour of the ant *Lasius niger* was studied in five cities in Poland and Germany
134 during the summers of 2020, 2021 and 2022 (Supplementary Table 1, Figure 1). The three
135 Polish cities were located along a latitudinal gradient, with Gdansk in the north, Warsaw in the
136 centre, and Cracow in the south. The two studied German cities were Berlin in the north and
137 Regensburg in the south. The studied cities differed in size and compactness; Berlin being the
138 largest one, followed by Warsaw, Cracow, Gdansk and Regensburg (Table 1).

139 The study plots in each city were selected according to the thermal structure of the urban heat
140 islands (UHIs). Therefore, in each city, we selected three urban parks close to the city centre
141 (Urban A, B and C – with a maximum impact of UHI) and three rural areas at the outskirts of
142 the cities (Rural A, B and C – with a lower impact of UHI) (Figure 1). Urban areas remained
143 brightly lit throughout the night due to the presence of streetlamps, while rural areas
144 experienced natural nocturnal illumination conditions. To ensure the presence of *L. niger*, we

145 searched for habitats whose characteristics matched the ecological preferences of this species.
146 All the selected plots shared the same landscape structure of lawns or grassland and similar
147 ground cover, sporadically including ornamental bushes (in urban parks) or herbaceous plants
148 (in rural areas).

149 **2.2. Field and laboratory studies**

150 **2.2.1. Field observations – Foraging activity at night**

151 To evaluate the influence of urban stressors (NW + ALAN) on ant activity, we measured the
152 nocturnal foraging activity of *L. niger*. Thus, we were able to capture only the effect of the
153 habitat temperature without the influence of sunlight. Following the ecological preferences of
154 *Lasius niger*, we specifically chose lawns located in urban parks or rural areas to ensure the
155 occurrence of this species. The sampled locations exhibited relatively similar vegetation cover,
156 with the presence of some herbaceous plants in rural habitats. This presence is notably absent
157 in urban areas, attributed to their aesthetic management practices. The observations were
158 conducted simultaneously in pairs of one urban park and one rural area (Supplementary Table
159 1). Linear arrays of bait stations consisting of a cotton ball soaked in sugar-water and a piece
160 of tuna were used. In each area, we placed two transects of ten baits every 5 m and a minimum
161 of 20 m between the two transects. We measured ground temperatures by placing three
162 thermometers along the transects on the ground surface (one at the beginning of the first
163 transect, one in the middle point between the two transects and the third at the end of the second
164 transect) (Figure 1).

165 After placing the baits at 1:30 a.m., we waited for 30 min to allow ant workers to locate the
166 food sources before data collection. The observations were performed in the middle of the night.
167 They started at 2 a.m. and were repeated every half hour for a total of 6 rounds of observations,
168 starting the last round of observations at 4:30 a.m. During each round, we recorded the number

169 of workers present at each bait and noted the temperature displayed on each thermometer. For
170 each site, the observations were repeated two days later, so that the observations took six nights
171 in total per urban and rural areas of one studied city.

172 **2.2.2. Laboratory experiment – Influence of the temperature and light pollution**

173 In field studies, ant activity can be influenced by factors other than temperature (i.e., humidity)
174 and evaluating the influence of light pollution is extremely difficult. Therefore, we carried out
175 an independent but complementary laboratory experiment to assess the effects of the urban
176 environment (NW and ALAN) on ant activity under controlled conditions. For the experiment,
177 we used *L. niger* “colonies” (a fragment of at least 500 workers and a minimum of 200 pieces
178 of brood) collected in Warsaw and Regensburg (Supplementary Table 2). In Warsaw, we
179 collected 18 “colonies” (9 urban and 9 rural ones) from the same three locations, in which the
180 field observations were performed. In Regensburg, the 18 “colonies” (9 urban and 9 rural) were
181 collected from two of the three urban and rural locations studied. From each “colony”, we
182 created three experimental colonies composed of 120 workers and 30 larvae of comparable size,
183 making a total of 54 experimental colonies from both Warsaw and Regensburg. These
184 experimental colonies were placed into artificial plaster nests, which had a nesting chamber at
185 the bottom and were connected to a foraging arena at the upper part of the structure by a vertical
186 tunnel in the plaster (Figure 1). Colonies were allowed to acclimatise to the new conditions for
187 two weeks (Sprenger et al. 2018, Schläppi et al. 2020) before the 8-day laboratory experiment
188 started. Experimental colonies were fed *ad libitum*, but using a feeding regimen established to
189 minimize disturbance. On the first day (*day 0*), we placed a small container with a solution of
190 sugar and water and a small metallic disk with tuna into the arena. We replaced the food in the
191 middle of the study period (*day 4*).

192 One experimental colony from each of the main original “colonies” was placed into each of
193 three distinct climatic chambers, each with unique temperature and light cycles: a *warm*
194 chamber simulated the urban temperatures (two thermal cycles: *high* 15 hours with light and 28
195 °C, *low* 9 hours of darkness and 17 °C), a *cold* chamber simulated the lower, rural temperatures
196 and the same light-dark cycles (*high* 15 h with light and 20 °C, *low* 9 h of darkness and 12 °C),
197 and a *light* chamber to test the effect of the light pollution (24 h with light, 15 h with 20 °C, 9 h
198 with 12 °C). Temperatures set in the climatic chambers were selected according to the average
199 of the maximum temperatures recorded in Warsaw during the sampling season. In each
200 chamber, we installed an infrared-enabled camera connected to a Raspberry Pi microcomputer
201 that was programmed to take a photo every half hour over the entire course of the experiment.
202 Later, we downloaded the photos and counted the number of workers present in each foraging
203 arena. The photos taken during *day 0* and *day 4* were not used to avoid activity changes linked
204 to the addition of food to the arena.

205 2.2.3. Colony growth

206 To evaluate whether the different environmental temperatures in urban and rural habitats
207 influenced colony development, we carried out a laboratory experiment. For the study, we
208 collected 18 “colonies” (fragments of at least 500 workers and a minimum of 200 larvae plus a
209 variable number of pupae) from urban areas and another 18 “colonies” from rural ones (6
210 colonies per each of the 3 study locations in Warsaw, Supplementary Table 1). Later, we set up
211 two experimental subcolonies from each “colony,” including 30 workers and 20 larvae into an
212 artificial nest made using a Petri dish. The original workers were marked on the gaster before
213 placing them in the nest to distinguish them from the newly emerging workers. Larvae were
214 weighed individually and only larvae with the same weight were selected.

215 For the study, we used a total of 36 experimental colonies from each urban and rural habitat (72
216 experimental colonies in total). Half of the experimental colonies (one from each above-
217 mentioned pair) were placed in the *warm* chamber (simulating urban temperatures - 15 h / 24
218 °C and 9 h / 17 °C), and the other half was placed in the *cold* chamber (simulating rural
219 temperatures - 15 h / 20 °C and 9 h / 12 °C.). In this experiment, we used slightly lower
220 temperatures in the warm chamber than in the previous experiment to reduce the thermal stress
221 in these nests in which individuals were strongly exposed to the environmental temperature,
222 given the absence of any insulating material in the artificial nests. Colonies were fed *ad libitum*
223 with an artificial diet (honey, agar, and egg) and mealworms through a small foraging arena
224 separating the food from the “nest”. Finally, the colonies were checked periodically 2-3 times
225 per week and on each day of observation we counted the number of living workers and the
226 number of emerging workers.

227 2.2.4. *Locomotory traits*

228 To check whether the differences between urban and rural habitats affect the locomotory
229 behaviour of ants, we tracked the movement of *L. niger* workers from 9 urban and 9 rural
230 “colonies”. The ant workers used in this study were obtained from the “colonies” collected in
231 Warsaw for the previous experiment. A total of 180 workers (ten workers per colony fragment)
232 were selected and placed individually into a cylinder located in the centre of a Petri dish (9 cm
233 diameter). To prevent the ants from escaping, we coated the walls of both the cylinder and the
234 Petri dish with fluon. To record the ants’ movement, we positioned a webcam (Webcam
235 A4Tech PK-910H) 30 cm above the Petri dish.

236 For the experiment, we placed one worker in the cylinder and waited for 1 min to allow the ant
237 to calm down after the manipulation. Then, we removed the cylinder and recorded the ant for
238 180 s. After the recording, the ant was placed in a plastic container. After the ten workers had

239 been tested, they were returned to their “colony” and the Petri dish was removed and cleaned
240 with alcohol to remove pheromones possibly deposited by the ants. We used a clean Petri dish
241 and a new cylinder for each observation. Later, we analysed the videos by tracking the ant
242 movement using the AnimalTA software (Chiara and Kim, 2023). We measured the proportion
243 of time the ant remained immobile, average speed, travelled distance, and exploration value
244 (proportion of the arena explored by the moving ant). Additionally, we also estimated the
245 boldness of the workers by calculating a score of thigmotaxis and the time of latency to get into
246 the central part of the arena. Thigmotaxis scores were calculated as the proportion of time spent
247 walking in the peripheral area of the arena. Therefore, higher thigmotaxis could be interpreted
248 as a lower boldness since increasing thigmotaxis is associated with aggregatory behaviour
249 (Depickère et al. 2008, Sanmartín-Villar and Jeanson 2022) and higher possibilities of hiding
250 from predators (Laurent Salazar et al. 2018). Similarly, a higher latency to enter the centre of
251 the arena can be interpreted as lower boldness of the worker.

252 3. Statistical analysis

253 Statistical analyses were conducted using R v4.1.2 (R core Team 2021) and RStudio (Posit
254 Team, 2023). Generalized linear models were carried out using the package *lme4* (Bates et al.,
255 2013) and graphical representation of the data was performed using the *ggplot2* package
256 (Wickham, 2016). The selection of the random factors included in the models was based on the
257 AIC scores obtained after model comparison using the function *anova*. We checked the
258 importance of the tested factors and interactions by carrying out an ANOVA of the models
259 using the functions *Anova.glmTBM* (for GLMM) from the *glmm.TBM* package, and *Anova*
260 (for LMM) from the *car* package (Fox and Weisberg 2019). Overdispersed and Zero-inflated
261 Poisson models were performed using the *glmmTMB* function from the *glmmTMB* package
262 (Brooks et al. 2017). The post-hoc tests for GLMM models were performed by using the
263 *emmeans* function from the *emmeans* package (Lenth 2023). Post-hoc pairwise comparisons

264 for LMM were calculated using the *posthoc_Pairwise* function of the *grafify* package (Shenoy
265 2021).

266 3.1. Field observations – Foraging activity at night

267 First, to get a general idea of the thermal trends during the nocturnal field observations, we
268 calculated descriptive statistics for the recorded temperatures. To visualise the dynamics of the
269 foraging activity in each city and habitat type, we constructed a column plot using the number
270 of occupied baits registered at each temperature during the whole period of observation per
271 study site (Supplementary Figure 1).

272 Thereafter, we assessed for differences in temperatures between urban and rural habitats using
273 LMM (Gaussian distribution). In the model, the average value of the three temperatures
274 registered per transect at each round of observations was used as the response variable and the
275 interaction of the habitat type (urban or rural) and the identity of the city were used as
276 explanatory factors. As nested random factors, we used the identity of the plot and the identity
277 of the day of sampling (first or second day). Later, a post-hoc pairwise comparison of the habitat
278 type per city was performed based on the estimated marginal mean.

279 To evaluate for differences in nightly foraging activity between urban and rural areas, we used
280 a GLMM (negative binomial distribution, family = *nbinom1*). In the model, since the number
281 of foragers was related to colony size, we used the number of baits occupied by ant foragers as
282 a response variable and the habitat type (urban or rural) as an explanatory factor. We used the
283 identity of the day of sampling (first and second day) and the identity of the plot (A, B, C) as
284 nested random factors. First, we performed the ANOVA of the model to check if the ant activity
285 was influenced by the habitat type and the city. Later, we performed post-hoc Tukey
286 comparisons for habitat type in each city with an estimated marginal mean obtained from the
287 *emmeans* package (Lenth 2023).

288 3.2. Laboratory experiment – Influence of temperature and light pollution

289 We tested the influence of temperature and illumination on ant activity by performing GLMMs
290 (Poisson distribution). First, we analysed the general influence of temperature and light
291 pollution on ant activity by carrying out the ANOVA of a model in which we used the number
292 of ants observed in the foraging arena in each photo as the response variable, the treatment
293 (*warm*, *cold*, and *light*) as an explanatory factor, and the identity of the experimental colony as
294 a random factor. Later, since the different temperatures related to the day/night cycles in the
295 setup may influence ant activity, we also tested the interaction of the treatment, the sections of
296 the treatments (day/night) and the habitat type where the colonies were collected by carrying
297 out an independent GLMM for each city (Warsaw and Regensburg). In the models, we used the
298 number of ants observed in the foraging arena in each photo as a response variable, the
299 interaction of the treatment, thermal section (*high* temperature or *low* temperature) and habitat
300 type (urban or rural) were used as explanatory factors, and the identity of the experimental
301 colony was used as a random factor. First, we tested the importance of the explanatory factors
302 and their interaction by carrying out the ANOVA of the model. Later, we tested the differences
303 in ant activity among treatments and sections by performing post-hoc Tukey comparisons on
304 the model. According to the setup of the different chambers, the effect of the temperature was
305 obtained by comparing the *warm* vs *cold* chambers, whereas the effect of illumination was
306 obtained by comparing the *cold* vs *light* chambers.

307 3.3. Colony growth

308 To check the differences in brood development between urban and rural habitats, we carried
309 out GLMMs. First, we tested with a GLMM (Zero-inflated Poisson) the differences in workers
310 emerging between the warm and the cold chambers, as well as the differences between urban
311 and rural habitats. In the model, the number of workers emerged was used as a response

312 variable. Later, we used a GLMM (Zero-truncated negative binomial model) to test for
313 differences in the mortality of workers by using the number of dead workers as a response
314 variable. In both models, the interaction of the treatment (identity of climatic chamber) and the
315 habitat type were used as explanatory variables and the identity of the experimental colony was
316 used as the random factor. Finally, to test for differences between urban and rural colonies, we
317 carried out a post-hoc test on the models.

318 *3.4. Locomotory traits*

319 To check whether the habitat type led to differences in the locomotory traits of the ant workers,
320 we carried out LMMs (Gaussian distribution). Since the values of the measured traits were
321 correlated, we analysed them in separate models. In the models, the value obtained for each
322 trait was used as a response variable and the habitat type (urban and rural) as an explanatory
323 variable. The identity of the worker and the identity of the colony were used as nested random
324 factors.

325 Moreover, we checked whether the different habitat types influenced the boldness of the ant
326 workers. We conducted two LMMs (Gaussian distribution) using the thigmotaxis scores and
327 the latency scores as the response variables. In both models, we used the habitat type as the
328 explanatory variable, and as nested random factors the identity of the worker and the identity
329 of the colony.

330 **4. Results**

331 We conducted a total of 1440 rounds of nocturnal observations with 720 rounds in urban areas
332 and an equal number in rural areas. We also analysed a total of 31,320 photos for the laboratory
333 experiment, half of which were obtained from the laboratory experiment in Warsaw and half
334 from Regensburg. This was composed of a total of 290 photos analysed per each experimental

335 colony in each climatic chamber during the six days of observation. Finally, a total of 180
336 workers (90 workers per habitat type) were tracked in the analyses of the locomotory traits.

337 *4.1. Field observations – Foraging activity at night*

338 Urban and rural habitats differed in the range of temperatures registered during the night
339 observations, differing around 8 °C between urban and rural habitats at the beginning of the
340 observations (Supplementary Figure 1). The minimum temperature registered in urban habitats
341 was 9.1 °C and the maximum was 23.4 °C, whereas in rural habitats it was 5.7 °C and 19.8 °C,
342 respectively. The difference between the maximum and minimum temperatures registered
343 during the observations was in general slightly higher in rural habitats (Table 1).

344 Temperatures during the night were warmer in urban areas than in rural ones ($t = 3.08$, $p =$
345 0.031). Although this trend is shared among all the studied cities, the strength of the differences
346 varied among them (Figure 2). Warsaw was the city showing the strongest differences in
347 temperature between habitat types ($t = -4.45$, $p = 0.019$), followed by Gdansk ($t = -3.96$, $p =$
348 0.025), Berlin ($t = -3.08$, $p = 0.044$) and Cracow ($t = -2.93$, $p = 0.050$). Regensburg was the
349 only city where the temperatures did not differ significantly between habitat types ($t = -1.45$, p
350 $= 0.232$).

351 Ant foraging activity was significantly higher in urban than rural areas ($\chi^2 = 4.02$, $p = 0.044$),
352 and varied across the cities ($\chi^2 = 57.72$, $p < 0.001$). The results show that the ant activity
353 registered during nights was influenced by the interaction of both the habitat type and the city
354 ($\chi^2 = 151.42$, $p < 0.001$; Figure 3). We found opposite trends in Warsaw and Regensburg: in
355 Warsaw, the activity was higher in urban areas than in rural ones ($z = 3.83$, $p = 0.005$), whereas
356 in Regensburg the higher activity was registered in rural areas ($z = -9.22$, $p < 0.001$). Differences
357 were not significant in Gdansk ($z = -2.29$, $p = 0.389$), Cracow ($z = 1.06$, $p = 0.987$) and Berlin
358 ($z = 2.36$, $p = 0.346$).

359 4.2. Laboratory experiment – Influence of temperature and light pollution

360 When testing the effect of temperature and light pollution on ant activity in chambers, the results
361 indicate a significant influence by both the treatment (*warm, cold, light*: $\chi^2 = 44.57$, $p < 0.001$)
362 and the origin of the colonies (Warsaw or Regensburg: 66.44, $p < 0.001$, Figure 4). Furthermore,
363 the interaction of these two factors showed to play an important role in the ant activity ($\chi^2 =$
364 14.07, $p < 0.001$) suggesting a variation of the response to the treatments set up in the chambers
365 between colonies from Warsaw and Regensburg (Figure 4). The observed activity was slightly
366 higher in the colonies from Regensburg in which the maximum value for the mean number of
367 active foragers was five, whereas in Warsaw the mean number was around one. Later, when
368 analysing ant activity separately for each city, the results of the ANOVA revealed that the
369 treatment significantly influenced the outcome of the experiments in both cities (Warsaw: $\chi^2 =$
370 29.83, $p < 0.001$; Regensburg: $\chi^2 = 38.96$, $p < 0.001$; Figure 4A, 4B). In colonies from Warsaw,
371 the general ant activity registered in the *warm* chamber was significantly higher than the one in
372 the *cold* chamber ($z = 4.38$, $p < 0.001$; Figure 4A). However, in Regensburg, the general ant
373 activity did not differ between the *warm* and the *cold* chambers ($z = 0.64$, $p = 0.987$; Figure
374 4B). Light had a positive influence on ant activity (*cold vs light*: $z = 6.17$, $p < 0.001$), although
375 the difference was only significant in the colonies from Regensburg (Regensburg: $z = 6.15$, p
376 < 0.001 ; Figure 4B) but not in Warsaw ($z = 2.18$, $p = 0.245$; Figure 4A).

377 We also carried out complementary analyses to check whether the strength of the response to
378 the increase in temperature or the light pollution differed between urban and rural populations,
379 as well as whether the response differed between thermal sections due to potential local
380 adaptations. Our results show that the influence of the thermal cycle, the habitat where the
381 “colonies” were collected (urban or rural) and the interaction of these two factors differed
382 between the treatments (climatic chambers) and the cities. For Warsaw colonies, both the
383 treatment and the interaction of the treatment and the thermal section had a significant influence

384 on ant foraging activity ($\chi^2 = 33.47$, $p < 0.001$; $\chi^2 = 7.53$, $p = 0.023$, respectively), but the
385 interaction of the thermal section with the treatment and the habitat type from where the
386 “colonies” were collected was not significant ($\chi^2 = 0.35$, $p = 0.837$). In Regensburg, the thermal
387 cycle ($\chi^2 = 3361.81$, $p < 0.001$), its interaction with the type of treatment ($\chi^2 = 101.36$, $p <$
388 0.001), as well as the interaction of the thermal cycle with type of treatment and the habitat type
389 from where the colonies were collected ($\chi^2 = 32.32$, $p < 0.001$) showed to influence the foraging
390 activity of the ants (Supplementary Figure 2).

391 When checking the trends in each climatic chamber (treatment) separately, we observed that
392 urban colonies from Warsaw tend to be generally more active than the rural ones in the *warm*
393 and *cold* chambers. No significant differences were found among treatments or between thermal
394 sections (Supplementary Table 3a, 3b; Supplementary Figure 2A, 2B). Colonies from
395 Regensburg differed from the pattern described for Warsaw colonies, and rural ants were more
396 active than the urban ones in all the treatments. Moreover, the ant activity was significantly
397 higher in the *warm* thermal cycle in all the treatments (Supplementary Table 3b, Supplementary
398 Figure 2C, 2D). Finally, no significant differences in activity were found between urban and
399 rural colonies, neither in the *high* or *low* thermal sections of the three treatments
400 (Supplementary Table 3a, Supplementary Figure 2).

401 3.3. Colony growth

402 The experiment lasted a total of 42 days, i.e., until all new workers had emerged in all the
403 colonies. Both the treatment ($\chi^2 = 26.53$, $p < 0.001$) and habitat type ($\chi^2 = 10.39$, $p = 0.001$), as
404 well as the interaction of these factors ($\chi^2 = 13.78$, $p < 0.001$) affected the larval development.
405 Significantly more workers emerged during the observation period in the experimental colonies
406 placed in the *warm* chamber ($z = 4.22$, $p < 0.001$). This trend was equally observed in colonies
407 coming from both habitat types (urban: $z = -4.74$, $p < 0.001$; rural: $z = -4.22$, $p < 0.001$).

408 Moreover, experimental colonies from urban areas showed a significantly lower acceleration
409 of the larval development than those coming from rural areas ($z = -4.33$, $p < 0.001$). Worker
410 mortality was influenced by the treatment ($\chi^2 = 17.61$, $p = 0.021$) and the interaction of
411 treatment and habitat type ($\chi^2 = 5.31$, $p < 0.001$). This indicates that the influence of habitat
412 type is linked to a particular treatment, but does not have a general influence ($\chi^2 = 0.65$, $p =$
413 0.418). The number of dead workers was significantly higher in the *warm* chamber ($z = 4.64$, p
414 < 0.001). This increase in mortality was significantly higher in the rural experimental colonies
415 ($z = -4.61$, $p < 0.001$) but not in the urban ones ($z = -1.31$, $p = 0.557$) (Figure 5).

416 3.4. *Locomotory traits*

417 Locomotory traits varied between ants collected from urban and rural colonies. In comparison
418 with rural ants, urban ants spent more time immobile ($t = 3.22$, $p = 0.001$), moved less quickly
419 ($t = -5.78$, $p < 0.001$), walked a shorter total distance ($t = -6.13$, $p < 0.001$) and were less
420 explorative ($t = -4.06$, $p < 0.001$). However, we did not find any significant differences in the
421 meandering of their pathway ($t = 1.01$, $p = 0.309$) or the proportion of time walking in the centre
422 of the Petri dish vs. its border: $t = 0.62$, $p = 0.535$) (Figure 6). Additionally, workers from the
423 two habitat types did not differ in their thigmotaxis scores ($t = 0.62$, $p = 0.535$). However,
424 workers from urban habitats took significantly longer to enter the central part of the arena ($t =$
425 2.53 , $p = 0.012$, Figure 7).

426 4. Discussion

427 By combining field and laboratory experiments, our study shows how the environmental
428 stressors of urban ecosystems impact ant functional traits. Our experiments revealed that NW
429 and ALAN both increase the ant foraging activity at night. The foraging patterns observed in
430 the field and the laboratory experiment also suggest that the influence of urban NW on foraging
431 traits might differ among cities with different structural characteristics (e.g., size), which

432 determine the local climatic conditions in which the ant population occurs. We also discovered
433 that although ant colony growth can benefit from higher temperatures, the potential for faster
434 growth might be counterbalanced by a higher worker mortality due to thermal stress. Moreover,
435 we also discovered that urban and rural ants may differ in their locomotor traits as a possible
436 result of facing different competitive pressures during foraging.

437 4.1. *Field study: How differ nocturnal temperatures and ant foraging activity between urban*
438 *and rural areas?*

439 In our study, temperatures registered during the night observations were generally higher in
440 urban areas than in rural ones. However, although most studies discuss UHI as a phenomenon
441 impacting all urban habitats, our results indicate that the degree of variation between the two
442 habitat types differed across cities, as previously suggested by Zhou et al. (2017). For instance,
443 we found greater differences between habitat types in larger and more compact cities such as
444 Warsaw, while the smallest and least densely populated city of Regensburg did not show any
445 significant difference among habitat types.

446 In our study, we hypothesised that NW and ALAN could facilitate ant foraging during the night.
447 Accordingly, our results show that the foraging activity of the synanthropic *L. niger* was
448 generally higher in urban habitats. However, the extraordinary plasticity of this opportunistic
449 species seems to have favoured its success in urban habitats (Konorov et al. 2017). Thus, urban
450 environmental conditions might induce a competitive advantage for this (and other)
451 synanthropic species by allowing them to exploit resources for a longer daily period. In contrast,
452 other species might be seriously negatively affected due to niche overlap or trophic network
453 rewiring (Tougeron and Sanders, 2023). For example, nocturnal ants, characterized by low
454 thermal tolerance and a lower hierarchical position (García-Robledo et al. 2018), could be

455 outcompeted by diurnal species. Consequently, nocturnal ants might struggle to thrive in such
456 a competitive scenario.

457 Environmental temperature is the most important factor regulating ant activity (Cerdá et al.
458 1988), and warm temperatures increase ant foraging. Accordingly, our study shows that ants'
459 response varied not only among habitat types but also among populations, and the increase in
460 the differences in ant activity between habitats was in line with the strength of the thermal
461 variation; again, the strongest differences were found in the warmest of the studied cities like
462 Warsaw.

463 *4.2. Laboratory experiment: How much does temperature and light influence ant foraging?*

464 Separating the effect of NW and ALAN in natural conditions is extremely difficult due to their
465 combined presence in urban environments. Therefore, we performed separate laboratory
466 experiments under controlled conditions. Our results showed differences in the response to
467 temperature both between urban and rural populations and also between Warsaw and
468 Regensburg ant populations. Various studies have previously reported differences among
469 populations, suggesting that the physiological traits of the workers might be shaped by local
470 environmental pressures (Willet et al. 2017, Shik et al. 2019, Tonione et al. 2020).
471 Interestingly, ants from Warsaw were more active in the warm climatic chamber, whereas the
472 opposite situation was found in ant populations from Regensburg, which were more active in
473 the cold climatic chamber. This activity pattern matches the patterns of temperature found in
474 these two cities. In our study, we also found that ants from Regensburg increased their activity
475 during the warm period; however, the activity of the ants from Warsaw showed a relatively
476 constant value between the two thermal sections. In this study, we also registered the same trend
477 observed in the field baits: a higher activity was recorded in urban colonies from Warsaw
478 whereas in Regensburg the higher activity was recorded for the rural colonies. An explanation

479 for these different responses between cities and among populations could be the result of the
480 adaptation to local thermal conditions. This result might suggest an evolutionary divergence
481 driven by the urban environmental pressures. Thus, colonies inhabiting warmer environments
482 develop a higher heat tolerance than their counterparts from cooler environments, i.e. rural
483 populations have higher sensitivity to summer temperatures in urban habitats (Martin et al.
484 2019).

485 Environmental illumination appears to modify some behavioural traits of ants (Chong and Lee
486 2009, Jones et al. 2019, Clifton et al. 2020) but its influence on foraging activity has not yet
487 been studied. The results of our study reveal that continuous illumination consistently leads to
488 higher ant activity. For instance, the highest activity in the whole experiment was registered
489 during the high-temperature period of the 24-hour illuminated chamber, demonstrating how
490 ALAN can work as an enhancer of NW in cities and can potentially increase the foraging ant
491 activity during nights.

492 *4.3. Does the UHI affect colony growth?*

493 Brood development is influenced by several factors including temperature. An increase in
494 temperature benefits colony size by reducing the developmental time (Molet et al. 2017),
495 although its impact varies among species or even populations (Penick et al. 2017). Accordingly,
496 we found that the number of workers emerging during the observation period was consistently
497 higher at warmer temperatures. Therefore, the higher temperatures of urban habitats would
498 favour colony growth, and consequently population growth, especially in opportunistic or heat
499 tolerant species (Menke et al. 2011). Surprisingly, although we thought that urban colonies
500 would experience a stronger increase of workers emerging at high temperatures and colonies in
501 colder habitats (rural) may be expected to evolve higher baseline developmental rate, we found
502 that rural colonies showed a stronger response to the increase of the temperatures. This counter

503 gradient response supports the findings of Penick et al. (2017), in which they document that
504 brood in urban colonies requires higher temperatures for development.

505 However, urban environments can also be dangerous since warmer environments increase the
506 risk of desiccation and thermal stress (Ślipiński et al. 2021). Accordingly, in our study, we
507 observed a higher worker mortality in the warm chamber than in the cold one. However, we
508 also observed a higher worker mortality in rural colonies, whereas no significant increase in
509 worker mortality was found between the cooler and warmer temperatures in the urban colonies.
510 Our findings are in agreement with those in Penick et al. (2017), in which they document that
511 urban workers have evolved higher resistance to the warmer temperatures; as well as with
512 Martin et al. 2021 in which they found that rural colonies experienced higher mortality than
513 urban ones during summer in urban habitats. However, while this trend may be observed among
514 generalist species, the impact of high temperatures could vary for more specialized species. For
515 example, populations of cold specialists might decline due to thermal stress, whereas hot
516 specialists might not be significantly affected by the warmer environment.

517 *4.4. Locomotory traits in urban and rural ant populations*

518 Changes in the landscapes due to urbanisation is also linked to changes in the structure of the
519 ant communities, their behaviour and mutualistic interactions (McKinney 2008, Miranda et al.
520 2013, Trigos-Peral et al. 2020, Rocha and Fellowes 2020). In our study, we found that habitat
521 type was associated with differences in the locomotory behaviour of the studied populations.
522 For instance, rural ants were found to be faster and more explorative than urban ones. These
523 findings could be explained by a lower foraging effort (competition for the monopolization of
524 resources) and lower risk of predation in more homogenous habitats like the urban environment
525 (Rocha and Fellowes 2020). However, our study was performed at a temperature of around 20
526 °C, a suitable foraging temperature for *Lasius niger* even if colonies developed in cooler

527 habitats, and the results could change at high temperatures. For example, Chick et al. (2019)
528 found a higher speed in urban colonies compared with rural ones at high environmental
529 temperatures. Notwithstanding, while ants showed differences in their response between urban
530 and rural habitats, it is not clear yet whether this is the result of a genetic divergence induced
531 by urban pressures or just behavioural plasticity (Sih et al. 2011, Miranda 2017, Jacquier et al.
532 2023). Nevertheless, this change in locomotory traits in urban populations may play a crucial
533 role in facilitating invasions in urban habitats. For example, Dáttilo and MacGregor-Fors (2021)
534 described how colonies of various invasive species, known for their adaptability in urban
535 environments, demonstrate a superior ability to locate resources compared to native colonies.
536 Therefore, this advantageous trait might enable the effective monopolization of resources,
537 potentially aided by the presence of less aggressive competitors in urban habitats. In fact,
538 similar to Jacquier et al (2023), we found that urban ants were less bold than rural ones. Despite
539 differences in study methodology, ant species ecology, and their hierarchy status, both studies
540 found that urban environments lead to less bold ant behaviour.

541 **5. Conclusions**

542 In this study, we showed for the first time how the combined influence of NW and ALAN
543 affects the foraging activity of *L. niger*, a synanthropic species used as a model organism in this
544 study. Urban night temperatures as well as light pollution were shown to facilitate foraging,
545 allowing colonies to broaden their foraging period; however, the trends might vary depending
546 on the strength of these environmental pressures. Moreover, our study also indicates that
547 workers from urban populations were slower, less explorative, and less bold, which might be
548 explained by weaker competitive pressures for resources in urban habitats. Additionally, urban
549 colonies showed lower worker mortality and faster brood development at higher temperatures,
550 which translates into ecological advantages relative to specialist species from cold
551 environments or species with low thermal plasticity. This has implications for understanding

552 the spread and success of opportunistic species, including invasive ones, under global warming
553 (Diamond and Martin 2021) and the continuous spread of urbanisation. Moreover, the results
554 of this study provide new insights into the role of NW, ALAN, and behavioural traits of native
555 urban populations, which might be involved in the urban biotic homogenization and invasion
556 success in human-modified habitats.

557 **AUTHOR CONTRIBUTIONS**

558 Project conceptualisation: GT, JH

559 Funding acquisition: GT, JH

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561 Data collection (laboratory): GT, SS, PC, MK, MW, ÁL

562 Experimental design (field): GT

563 Experimental design (laboratory): TJC, GT

564 Video and picture analysis: SS, GT, PC, MW

565 Statistical analysis: GT

566 Writing (first draft and revised version): GT

567 Writing (editing): all authors

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581 **CONFLICT OF INTEREST STATEMENT**

582 The authors declare no conflict of interest.

583 **DATA AVAILABILITY STATEMENT**

584 The complete dataset on which this study is based, as well as the entire statistical code, and
585 output generated by it, are available in figshare scientific repository at:
586 <https://figshare.com/s/b45473e80bfa0c2c3efa> . Doi: 10.6084/m9.figshare.23507211

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796

797 **Figure Captions**

798 **Figure 1.** Graphical abstract. Illustrations designed and drawn by Sergio Ibarra.

799 **Figure 2.** Boxplot representation of the temperatures registered during the night observations 800
in different cities and habitat types. Below: representation of the trends of the average 801
temperatures registered across the plots of the same habitat type during the field observations 802
performed at night in different cities and habitat types. The Y-axis represents the values of the 803
temperatures measured in each round of observations, while the X-axis shows the identity of 804
the observation round throughout the night sampling.

805 **Figure 3.** Violin plot representation of the number of baits occupied with *L. niger* foragers 806
during the field observations during the night in different cities and habitat types. The dot in the 807
centre indicates the mean and the line corresponds to the 95% confidence interval of the 808
collected data.

809 **Figure 4.** Mean and 95% confidence interval representation of the number of active foragers 810
registered in the laboratory experiment using climatic chambers. The two cities (Warsaw and 811
Regensburg) from where the colonies were collected are shown separately. Figures 4A and 4B 812
represent the differences in mean ant activity across the different treatments for urban and rural 813
populations from Warsaw and Regensburg.

814 **Figure 5.** Mean and 95% confidence interval representation of the number of workers emerged 815
and the number of dead workers registered in the laboratory experiment using climatic 816
chambers.

817 **Figure 6.** Violin plot representation of the locomotory traits registered in urban and rural
818 populations of *Lasius niger* from Warsaw. Within each plot is represented the mean and 95%

819 confidence interval of the collected data. Only the traits showing significant differences
820 between the two populations were included.

821 **Figure 7.** Raincloud plot representation of the time of latency spent to walk for the first time
822 into the centre of the arena registered in urban and rural populations of *Lasius niger* from
Warsaw. The figure on the right side of the plot corresponds to the violin plot representation of
824 the data. The figure on the left side of the plot corresponds to the representation of the data in
825 which each dot corresponds to one measurement. In the centre, data are represented as a boxplot
826 in which the line in the middle of the box is the median; the upper and bottom sides of the box
827 are the Q2 and Q3, respectively; and the upper and bottom whiskers represent the maximum
828 and minimum values of latency, Q1 and Q4, respectively.

829 **Supplementary Figure 1.** Graphical representation (mirroring column plot) of the number of
830 baits occupied by foragers of *Lasius niger* at each temperature during the nighttime field
831 observations in urban and rural habitats of the five studied cities.

832 **Supplementary Figure 2.** Figures 2A and 2B represent the differences in mean ant activity
833 between the *warm* and *cold* treatments, as well as the differences in the mean ant activity
834 between colonies collected from different habitat types during each thermal section. Figures 2C
835 and 2D represent the differences in mean ant activity between the *cold* and *light* treatments, as
836 well as the differences in the mean ant activity between colonies collected from different habitat
837 types during each thermal section. The origin of the colonies has been represented in green for
838 colonies collected from rural areas and yellow for colonies collected from urban areas. The
839 pairwise comparison rural-urban per each treatment is represented by a line connecting the two
840 mean values. The connecting line is red for the colonies submitted to the *warm* treatment, blue
841 for the colonies submitted to the *cold* treatment and yellow for the colonies submitted to the
842 *light* treatment.

Table 1. Data table including the characteristics of the city in terms of size, population density and temperatures. Maximum, minimum and mean temperatures (°C) were registered during the field observations during the nighttime in the two habitat types of the five studied cities. In the last column, the differences between minimum and maximum temperature values are reported.

	City	Population characteristics		Temperature (°C)			
		Area (km ²)	Population density (inhabitants/km ²)	Minimum	Maximum	Mean	Difference Max - Min
Rural	<i>Berlin</i>	-	-	6.97	19.8	15.51	12.83
	<i>Warsaw</i>	-	-	5.7	19.27	13.64	13.57
	<i>Cracow</i>	-	-	12.6	17.7	14.64	5.1
	<i>Gdansk</i>	-	-	13.53	17.5	15.31	3.97
	<i>Regensburg</i>	-	-	7.73	17.17	14.23	9.44
Urban	<i>Berlin</i>	891	3845.5	14.3	23.4	18.19	9.1
	<i>Warsaw</i>	517	3292.3	13.33	21.63	17.54	8.3
	<i>Cracow</i>	327	2309	15.07	20.83	17.21	5.76
	<i>Gdansk</i>	263	1753.4	9.15	21	18.75	11.85
	<i>Regensburg</i>	80	1599.2	12.4	17.27	15.5	4.87

Supplementary Table 1. Sampled locations during the field study in urban and rural areas in Poland and Germany.

	Urban	GPS coordinates	Rural	GPS coordinates
Berlin	Volkspark Hasenheide	52.483137, 13.415994	Pumptrack Berlin Spandau, Gatow	52.473847, 13.133658
	Charlottenburg	52.521584, 13.292882	Lübars	52.614334, 13.372726
	Mauer park	52.542953, 13.401601	Falkenhagener Feld	52.476024, 13.127250
Warsaw	Ogród Saski Park	52.240482, 21.008921	Gmina Michałowice	52.159636, 20.873293
	Morskie Oko Park	52.205943, 21.026827	Klaudyn	52.272952, 20.861246
	Marshal Edward Rydz-Śmigły Park	52.225446, 21.033746	Warsaw Airport	52.146279, 20.987392
Cracow	Barbakan Krakowski	50.065359, 19.940869	Bronowice	50.070671, 19.876318
	Henryka Jordana Park	50.063552, 19.915477	Prądnik Czerwony	50.102564, 19.984757
	Planty Dietlowskie	50.054007, 19.943469	Na Niwach	50.053494, 20.045647
Gdansk	Dariusza Kobzdej	54.352566, 18.650453	Olszynka	54.339410, 18.679444
	Park Kalisteniczny	54.356185, 18.657831	Kowale	54.313574, 18.539758
	Plac Zabaw	54.350082, 18.671136	Matarnia	54.383480, 18.475440
Regensburg	Dörnbergpark	49.015840, 12.085530	Universitätsklinikum, west	48.988750, 12.084810
	Stadtpark	49.018830, 12.078860	Pürkelgut	49.001988, 12.129880
	Ostpark	49.008454, 12.116981	Schönhofen	49.012830, 11.963030

Supplementary Table 2. Sampled locations for the laboratory studies in urban and rural areas in Warsaw and Regensburg.

	Urban	GPS coordinates	Rural	GPS coordinates
Warsaw	Ogród Saski Park	52.240482, 21.008921	Gmina Michałowice	52.159636, 20.873293
	Morskie Oko Park	52.205943, 21.026827	Klaudyn	52.272952, 20.861246
	Marshal Edward Rydz-Śmigły Park	52.225446, 21.033746	Warsaw Airport	52.146279, 20.987392
Regensburg	Dörnbergpark	49.015840, 12.085530	Universitätsklinikum, west	48.988750, 12.084810
	Stadtpark west	49.018830, 12.078860	Universitätsklinikum, east	48.988750, 12.084810
	Stadtpark east	49.018990, 12.084090	Schönhofen	49.012830, 11.963030

Supplementary table 3. Results of the GLMMs performed on the ant activity data collected during the laboratory experiment in the climatic chambers to compare the activity between populations between habitat types in each treatment (Table 2a) and to compare the activity of each population between thermal sections of each treatment (Table 2b). Significant results are highlighted in bold and significant p-values marked with an asterisk. The treatments and the thermal sections of each treatment are the following ones: *warm* treatment (*high* section -15 hours- with light and 28 °C + *low* section -9 hours- of darkness and 17 °C), *cold* treatment (*high* section -15 h- with light and 20 °C + *low* section -9 h- of darkness and 12 °C), and a *light* treatment (*high* section -15 h- with 20 °C + *low* section -9 h- with 17 °C).

Table S3a: ant activity rural vs urban population

		Habitat comparison			
		Warsaw		Regensburg	
Treatment	Comparison	<i>z</i>	<i>p</i>	<i>z</i>	<i>p</i>
Warm	Rural vs Urban: <i>high section</i>	-0.51	0.986	1.10	0.686
	Rural vs Urban: <i>low section</i>	-0.78	0.861	1.28	0.572
Cold	Rural vs Urban: <i>high section</i>	-2.97	0.015*	1.33	0.540
	Rural vs Urban: <i>low section</i>	-2.47	0.064	0.95	0.774
Light	Rural vs Urban: <i>high section</i>	0.82	0.841	0.75	0.873
	Rural vs Urban: <i>low section</i>	1.05	0.719	0.69	0.899

Table S3b: ant activity high vs low section

		Section comparison			
		Warsaw		Regensburg	
Treatment	Comparison	<i>z</i>	<i>p</i>	<i>z</i>	<i>p</i>
Warm	Rural: high vs low	0.36	0.983	10.39	> 0.001*
	Urban: high vs low	1.48	0.446	11.09	> 0.001*
Cold	Rural: high vs low	0.92	0.791	19.5	> 0.001*
	Urban: high vs low	1.96	0.201	13.69	> 0.001*
Light	Rural: high vs low	0.30	0.990	15.43	> 0.001*
	Urban: high vs low	-1.05	0.719	14.19	> 0.001*

Figure 1

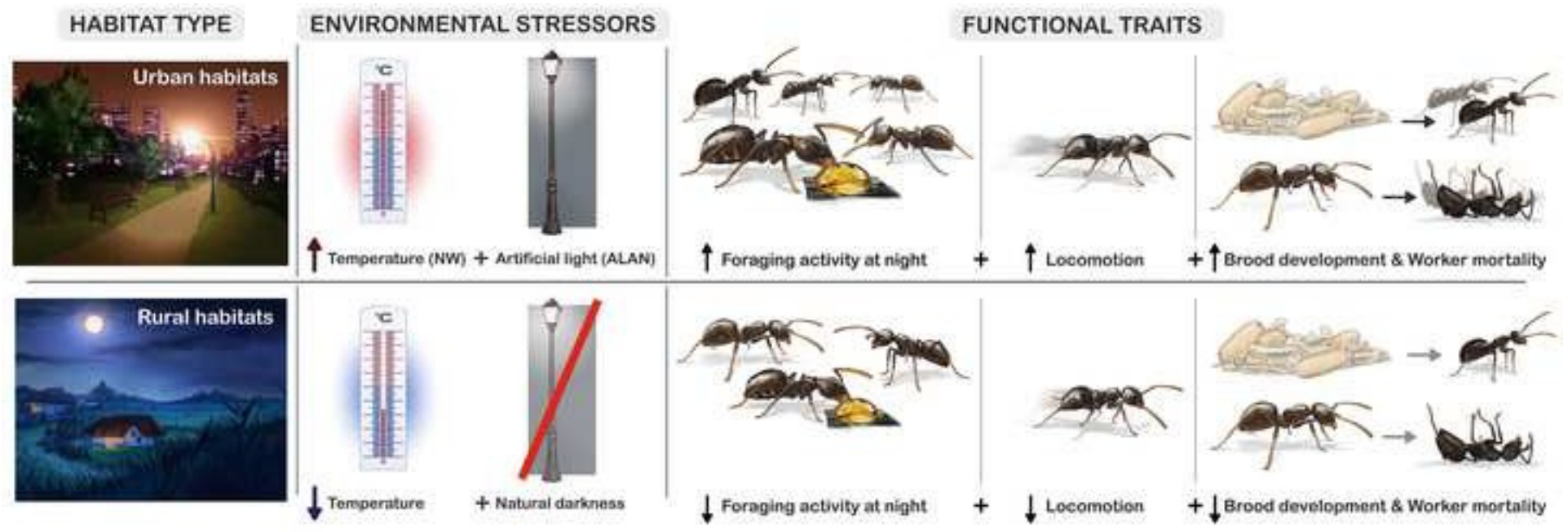


Figure 2

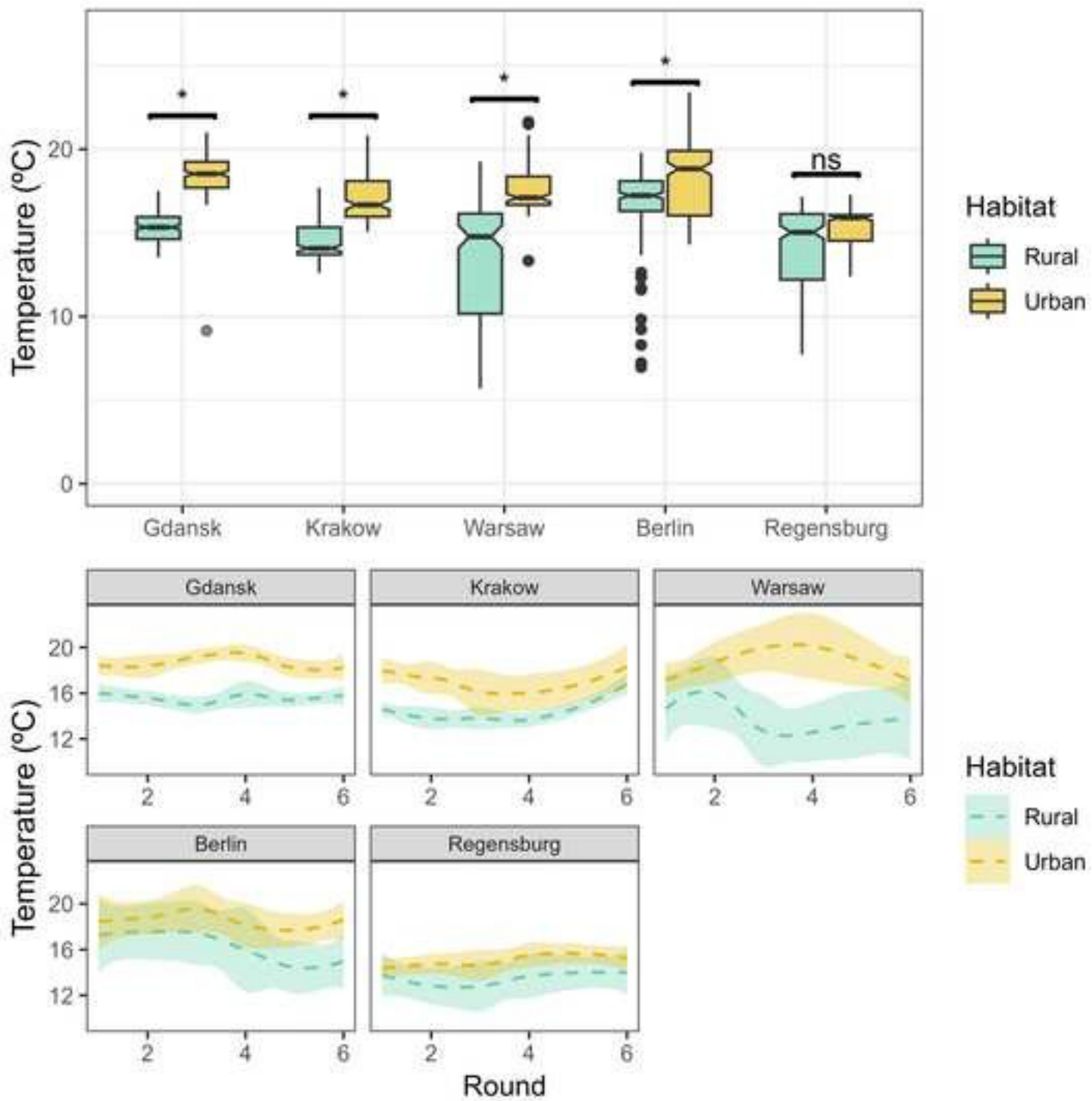


Figure 3

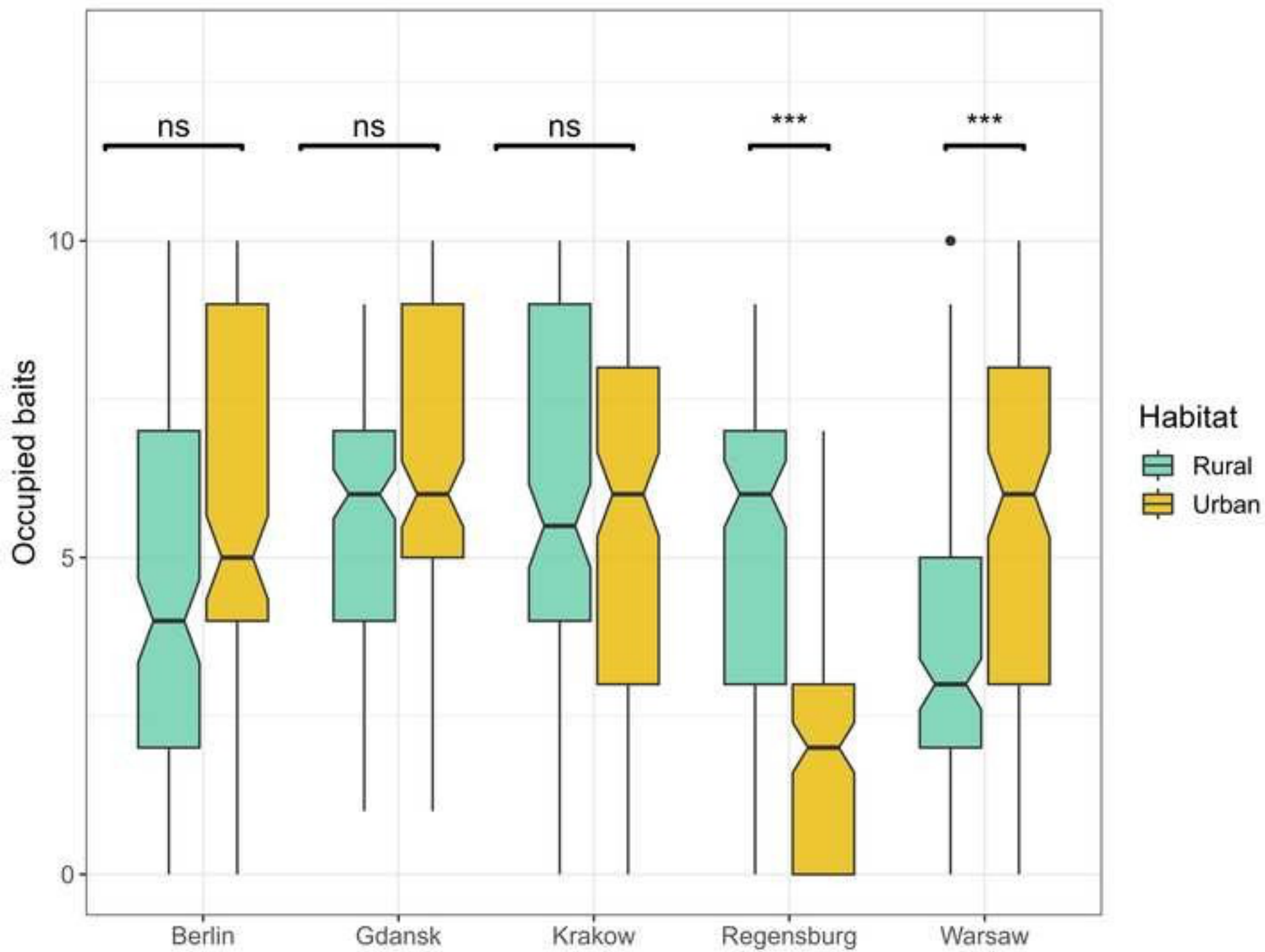


Figure 4



Habitat ◆ rural ◆ urban

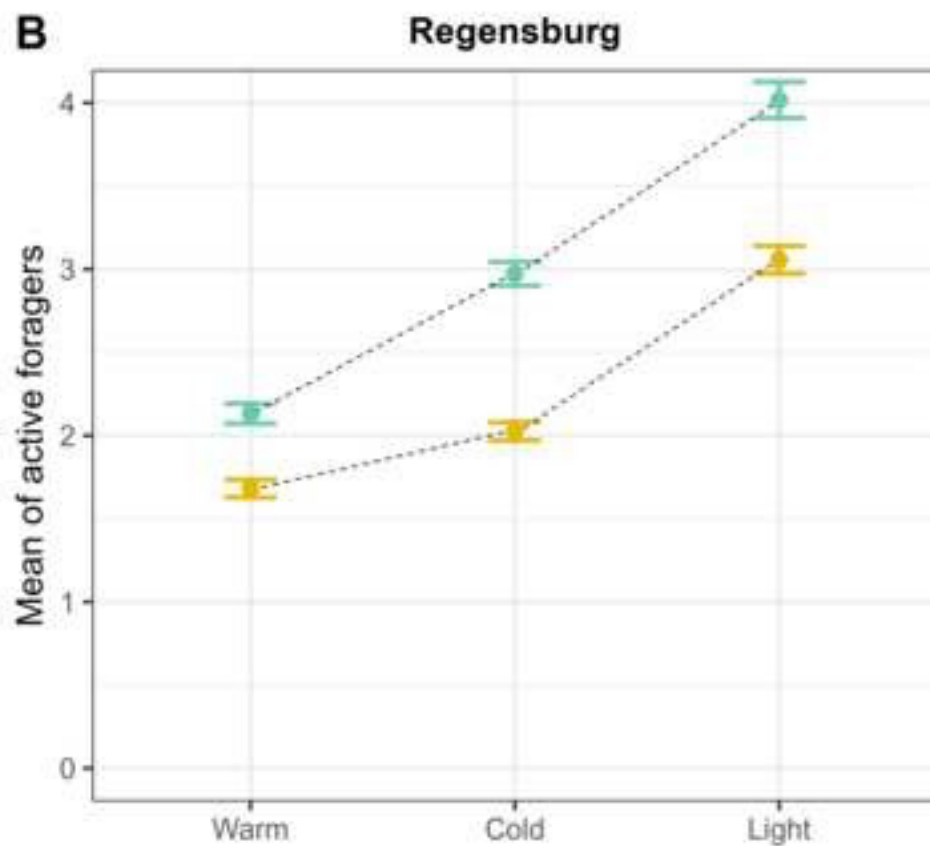
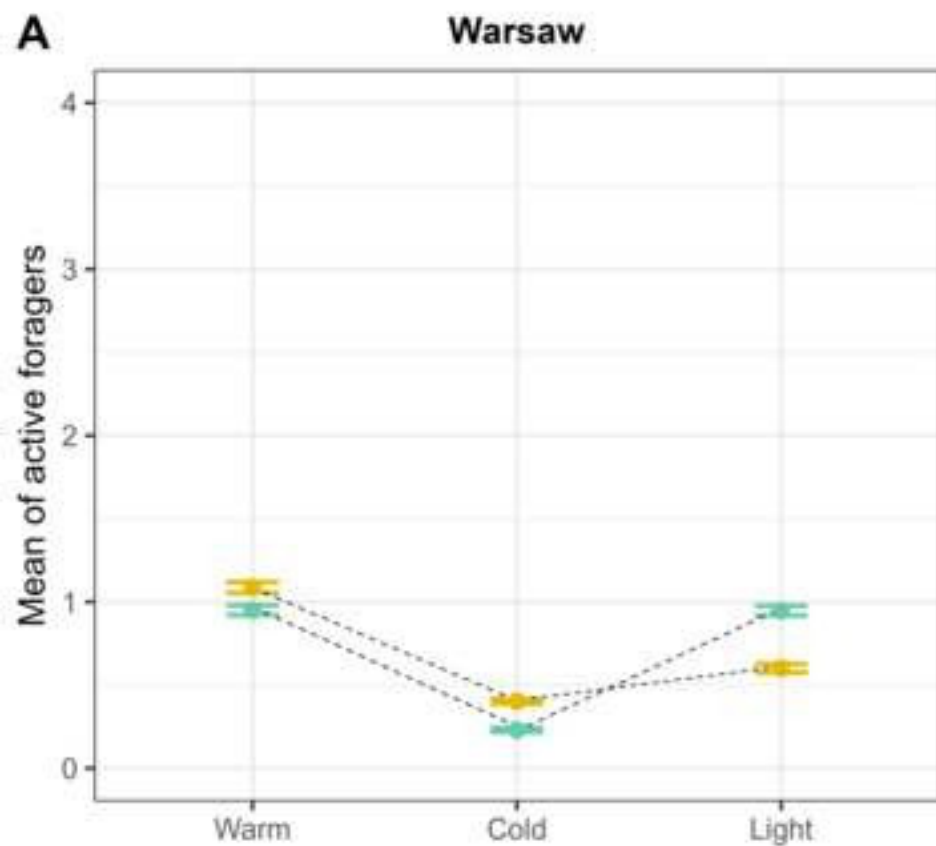


Figure 5

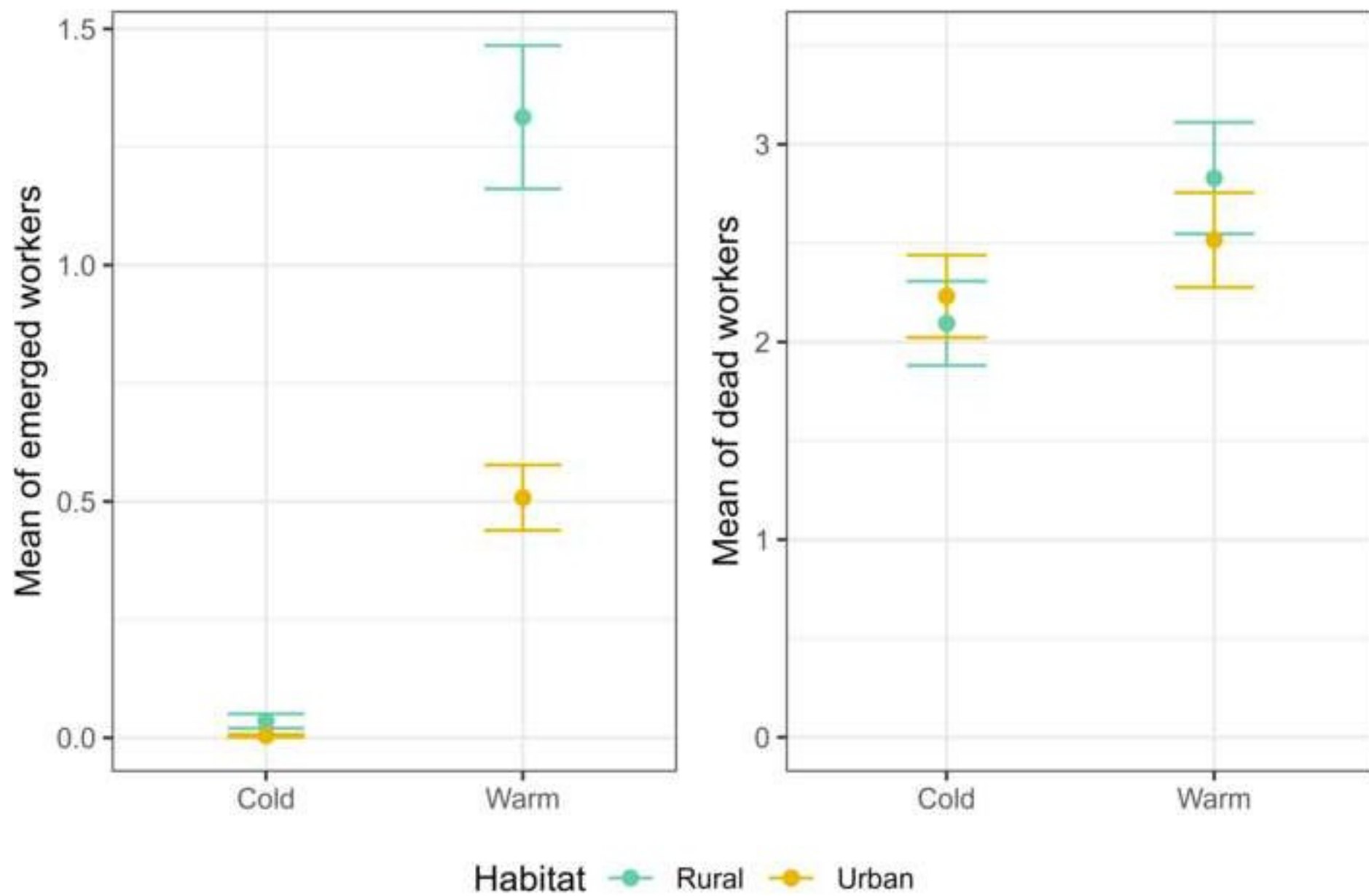


Figure 6

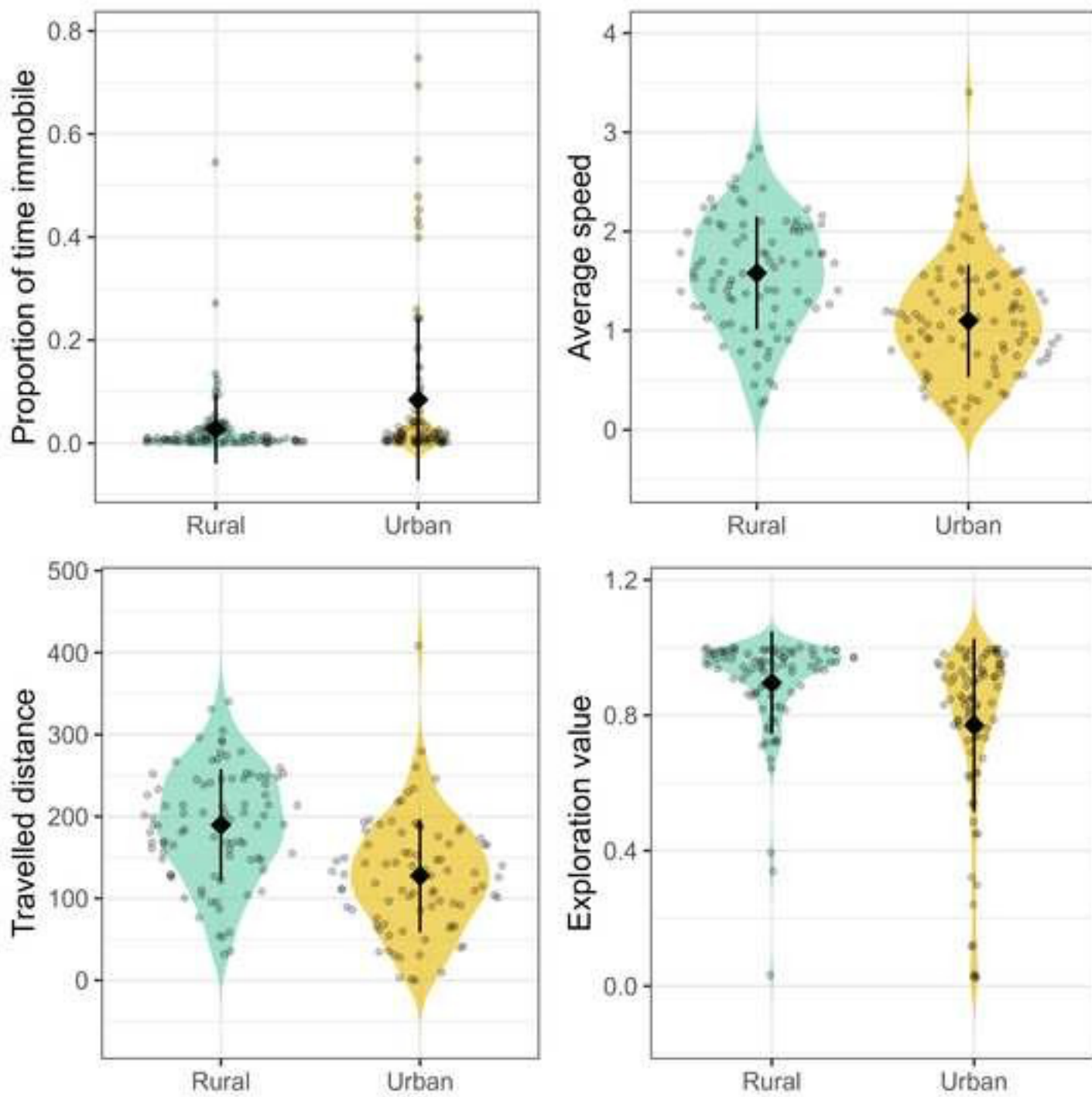


Figure 7

