

## Supplementary material

S1. Extended version of Methods section; S2. Timing of the experiments conducted in each of the studied populations; S3. PCA excluding data from feeding innovation experiment to increase sample size; S4. Table describing observations from the VES; S5. Body size, body condition, and behavioral traits; S6. Effect sizes; S7. Effects of the presence of *L. carinatus* on foraging behavior of *A. sagrei*. S8. Cartoon representation of the experimental setup described in the Methods section.

### **S1. Extended version of Methods section** (citations can be found in main text)

#### *1. Study species and populations*

Brown anoles (*Anolis sagrei*) are small lizards that spend most of their time perched low on tree trunks (up to ~1.5m) and on the ground [39]. When perching, lizards receptive to feeding usually assume a survey posture – head down, forequarters lifted off the substrate, and hind limbs extended backwards up the vertical tree trunk – while scanning the ground for potential prey, primarily arthropods [39,45,46]. They tend to abandon this posture when fed to satiation [45,46]. Larger predatory curly-tailed lizards (*Leiocephalus carinatus*) spend the vast majority of their time on the ground [47] and eat *A. sagrei* in the areas where they coexist [40,44]. Importantly, *A. sagrei* established in our study areas in Broward and Dade counties between 1951 and 1964 [48], whereas *L. carinatus* was first documented in urban areas decades later, from the early 2000's, although exact dates are unknown [49].

The presence of *L. carinatus* has been shown to increase the perch height of *A. sagrei* and they are a strong agent of natural selection for *A. sagrei* [42,43,50]. We measured the behaviour of lizards from two replicate populations of each of three different habitat types: two populations of forest-dwelling lizards, two urban populations with *L. carinatus*, and two urban populations without *L. carinatus*. We studied the effects of *L. carinatus* presence solely in urban environments because, to

32 our knowledge, there are no similar forested habitats with *L. carinatus* lizards  
33 present in the range of *A. sagrei* in South Florida. We captured 18-20 male *A. sagrei*  
34 lizards from each of these six sites. All lizards were captured and experiments  
35 conducted in June and July 2015.

36

37 Sites and their coordinates are indicated below:

| Sites          | Coordinates |            |
|----------------|-------------|------------|
|                | North       | West       |
| Gables         | 25.744259   | -80.257023 |
| Fern forest    | 26.226916   | -80.189077 |
| Las Olas       | 26.117793   | -80.111504 |
| Library        | 26.121980   | -80.261743 |
| Markham forest | 26.133741   | -80.349060 |
| South Miami    | 25.701427   | -80.295733 |

38

### 39 *2. Fieldwork methods and escape behavior*

40 First, we located a lizard perched between 20-200 cm on vegetation, artificial poles  
41 or walls. We slowly approached the lizard with an outstretched arm holding a laser  
42 distance meter. The meter was used to measure the escape distance (also known as  
43 flight initiation distance, FID), which is the distance from the observer to the lizard  
44 at the time the lizard initiates escape, moving at least one body length. To prevent  
45 the possibility that some behavioral types were captured more often than others  
46 (e.g. more exploratory lizards could be more exposed whereas shier lizards could  
47 escape more often), two researchers participated in the capture of the lizards after  
48 escape distance was measured, which ensured our capture rates were very high. We  
49 subsequently caught each lizard using a noose and quantified its aggressiveness at  
50 capture by counting the number of times the lizard threatened with its mouth open  
51 (termed gaping) when we gently moved the noose to its snout 10 times in 20  
52 seconds. Finally, we moved the lizard into an experimental cage and began the field-  
53 based experiment within 15 minutes after capture.

54

### 55 *3. Experimental set up and field experiments*

56 We used collapsible cages (68.5 X 68.5 X 122 cm) to conduct behavioural trials in  
57 the field. Cages had a transparent plastic front and three sides with mesh, which  
58 prevented small insects from entering the cages. We evenly distributed six 30-cm  
59 high, 1.5-2 cm wide perches made of branches collected from our study sites within  
60 each cage. We installed a square, wooden refuge (25 X 23 X 18 cm wide, long and  
61 high respectively) at one end of the cage. It had a semicircular 9-cm opening at the  
62 front, which was initially covered by a thick, dark cloth. All trials were filmed using a  
63 camera on a tripod ~2 m from the experimental cages. The researcher sat  
64 motionless at a distance of >5 m from the experimental cage during both the field  
65 and captivity trials, which is more than twice the mean FID for lizards in the habitat  
66 type that had longer FIDs. Both our preliminary trials and previous studies [51,52]  
67 showed this distance was enough for lizards to act normally so that field  
68 behavioural observations could be obtained.

69

70 *Exploration experiment.* We gently placed the lizard inside the refuge.  
71 Following previous studies [53], we used a habituation time of 3 minutes, after  
72 which we uncovered the opening of the refuge by gently pulling a string tied to the  
73 piece of cloth covering the refuge. In a few cases, lizards were found to be perched  
74 on the cloth at the beginning of the experiment. These trials were removed from the  
75 analyses. Maximum experimental time was 30 minutes based on results from  
76 preliminary trials using different lizards. Once the full body of the lizard was outside  
77 the refuge, we allowed it to explore for 15 minutes before terminating the  
78 experiment. Thus, total experimental time exceeded 30 minutes when lizards  
79 emerged from the refuge between 15 and 30 minutes. The following behaviours  
80 were measured (in seconds) from videos of the trials: i) 'Time to Head Out' was the  
81 time when the lizard first poked its head out of the refuge (i.e. eyes aligned with the  
82 entrance of the refuge); ii) 'Time to Body Out' was the time when the lizard's full  
83 body was out of the refuge (i.e. all four limbs fully out from the line of the entrance;  
84 not including the tail); iii) 'Time to First Perch' (i.e. all four limbs on the perch); iv)  
85 'Visual exploration time' was computed as (Time to Body Out – Time to Head Out);  
86 v) 'Time exposed' was obtained from subtracting (Time to First Perch – Time to

87 Body Out); and lastly, vi) the ‘Total number of perches’ used during 15 minutes as a  
88 surrogate for spatial exploration. Anoles were then individually kept in containers at  
89 room temperature in the dark for two days until captivity experiments were  
90 conducted.

91

#### 92 *4. Experiments in captivity*

93 *Exploration and attack simulation (boldness) experiments.* Exploration  
94 experiments described above were repeated in the lab two days after field  
95 experiments with the addition of the following procedures. When lizards were  
96 outside their refuges in the cage at the end of the 15-min exploration time, a  
97 researcher (OL) slowly approached the cage and gently chased them in the direction  
98 of the refuge by using the tip of the noose with which we had captured them covered  
99 with a red cloth. Lizards that did not emerge from the refuges during the exploration  
100 trial could not be tested for the attack simulation trial (n = 22). Time to hide inside  
101 the refuge was measured as number of seconds from the moment we opened the  
102 cage zipper until we closed it, after the lizard got in its refuge. The researcher then  
103 returned to his initial position >5 m from the cage and measured again the latency  
104 for lizards to poke their head out, body out, and time to climb the first perch. The  
105 trial was then terminated and lizards returned to their individual containers.  
106 Finally, we repeated the full exploration and attack simulation protocols two days  
107 later for lizards from three populations (one from each habitat type) to assess for  
108 repeatability of behaviours in captivity.

109 *Foraging innovation experiment in captivity.* We tested lizards’ willingness to  
110 feed in a novel context in captivity. We placed a novel experimental feeding tray  
111 containing 10 mealworms in the experimental cage and measured the latency to  
112 capture the first mealworm in the feeding tray. Based on results obtained from  
113 preliminary tests for lizards from other populations, a time limit of 15 minutes was  
114 set, after which they were assigned a conservative maximum time of 901 seconds.  
115 We conducted these trials using lizards from three of six populations (one replicate  
116 from each habitat type; n = 50).

117

118 *5. Estimation of within-individual consistency and behavioural syndromes*

119 Individual consistency in behavioural traits (i.e., animal personalities for a given  
120 behavioural trait at different times and in different contexts) was estimated by using  
121 intra-class correlation indices (ICC) using the package {irr} [54] available in the R  
122 platform (R core team). Repeatability tests were conducted for the time spent in a  
123 refuge before emerging to a new environment, time spent exploring visually before  
124 emerging from the refuge, spatial exploration as the number of perches visited,  
125 latency to emerge from refuge after a simulated predator attack, and time to hide  
126 inside the refuge after the simulated attack. Description of behavioural syndromes  
127 (i.e., the correlation among different behavioural traits estimated from different  
128 observational or experimental approaches) was based on a Principal Component  
129 Analysis in which each PC axis represents an independent axis of behavioural  
130 variation, representing behavioural variation at the population level. We conducted  
131 a PCA by using a correlation matrix after scaling the scores of all variables to have  
132 unit variance using the “prcomp” function in R (R core team).

133

134 *6. Daily patterns of activity*

135 *Visual Encounter Surveys (VES)* were conducted to measure lizard activity  
136 and habitat use throughout the day. A VES was conducted in each half-hour time  
137 block from 0630 to 2030 for a total of 28 surveys in each of the six populations in  
138 June 2015. In each of these 168 surveys, the same observer (ZC) walked the same  
139 section of a habitat at a constant pace and used a stopwatch to standardize effective  
140 observation time to 10 minutes (i.e. the time in which the observer was actively  
141 searching for lizards, discounting time in which the observer was recording data).  
142 Therefore, each VES typically took ~20 minutes. During the surveys, we recorded  
143 the number, species, age (including hatchlings), and sex of lizards as well as their  
144 perch height and diameter. *Anolis sagrei* represented 95% of the observations  
145 across all populations (Supplementary Material S4). Based on the observations from  
146 the VES, we examined daily patterns of activity for each *A. sagrei* population. The  
147 number of *A. sagrei* lizards on the ground was used as a surrogate of foraging  
148 activity (see Drakeley *et al.* 2015).

149

150 *7. Risk-taking analysis in free-ranging lizards*

151 We assessed whether foraging behaviour in the wild was consistent with  
152 divergent patterns of *A. sagrei* foraging activity found in captivity for lizards from  
153 urban habitats with and without predatory *L. carinatus*. We assessed the willingness  
154 of lizards to feed on new food resources presented. The experimental design  
155 consisted of presenting a live mealworm on the ground at a distance of ~1 m from  
156 an *A. sagrei* perched in survey posture. We conducted experimental trials at three  
157 different times of the day: early morning (within two hours after dawn), midday-  
158 afternoon (1230-1600 hrs), and evening (within two hours before dusk). We  
159 measured lizards' latency to descend to the ground and feed on the mealworm  
160 ('Latency to feed') as well as the time spent on the ground after first biting the  
161 mealworm ('Time on ground'). At least 30 trials were conducted for each time  
162 period at each of the four urban sites (n = 384 observations). We collected data on  
163 perch height, type, and diameter. Moreover, to investigate the direct effect of the  
164 instantaneous presence of a predator on *A. sagrei* foraging behaviour (latency to  
165 feed and time on ground), we noted whether there was a *L. carinatus* present within  
166 10 m of the focal lizard. Finally, we compared measurements between habitats with  
167 and without predators restricting to observations where there was no *L. carinatus*  
168 present. Our aim was to understand the degree of plasticity versus intrinsic  
169 differences in foraging decisions. We conducted these analyses during the midday-  
170 afternoon time block when *L. carinatus* were primarily active.

171

172 *8. Statistical analyses*

173 Differences among populations were tested using ANOVA and Tukey's Honestly  
174 Significant Difference (HSD) *post hoc* tests when data were normally distributed,  
175 including the analysis for differences between PC scores of lizards from different  
176 habitat types. We also used ANOVAs and Tukey's HSD tests to assess differences  
177 between foraging trials in the wild among time periods and habitat types. After  
178 pooling together both replicates for each habitat type, two-sample non-parametric  
179 Kolmogorov-Smirnov tests were used to evaluate whether patterns of lizard ground

180 use throughout the day differed among habitat types. Finally, we used {survival} R  
181 package [56] to analyze right censored data obtained from experiments in which a  
182 maximum time limit was set. The Kaplan-Meyer non-parametric method allowed us  
183 to estimate the survival function and build survival curves for each condition (e.g.  
184 habitat type, time blocks). We tested the significance of different survival curves by  
185 using the G-rho family rank test for censored survival data [57]. Finally, we  
186 investigated if morphology (SVL, body mass and body condition) played a role in  
187 determining the behaviours of *A. sagrei* (see Supplementary Material S1).

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189

190 **S2. Timing of the experiments conducted in each of the studied populations**  
 191 Numbers embedded in the table correspond to the number of replicates (i.e. sites) of  
 192 each treatment category for which we conducted each experiment.

193

|                                    | Wild   |             | Captivity   |                   |             |                   |                     |
|------------------------------------|--------|-------------|-------------|-------------------|-------------|-------------------|---------------------|
|                                    | Day 1  |             | Day 3       |                   | Day 5       |                   |                     |
| Habitat type                       | Escape | Exploration | Exploration | Attack simulation | Exploration | Attack simulation | Foraging innovation |
| Urban w/out<br><i>L. carinatus</i> | 2      | 2           | 2           | 2                 | 1           | 1                 | 1                   |
| Urban with<br><i>L. carinatus</i>  | 2      | 2           | 2           | 2                 | 1           | 1                 | 1                   |
| Forest                             | 2      | 2           | 2           | 2                 | 1           | 1                 | 1                   |

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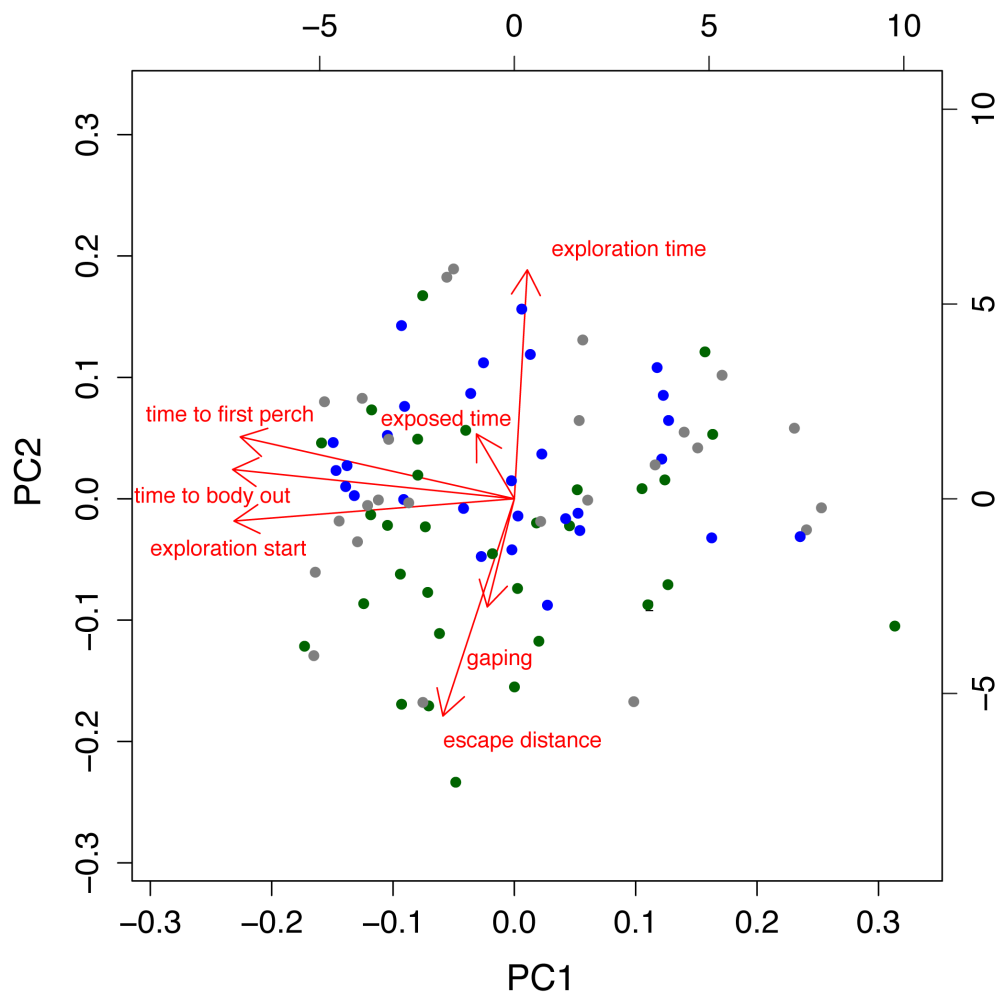
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**S3. PCA excluding data from feeding innovation experiment to increase sample size.**

PC1 and PC2 are shown in the figure. Note that PC4 in this plot differs from the plot in the main text because feeding innovation is not included in this PCA. However, note that both PC1 and, most importantly, PC2 in this expanded analysis are very similar to the one from the main paper.

|                          | PC1      | PC2      | PC3      | PC4      |
|--------------------------|----------|----------|----------|----------|
| Escape distance          | -0.18063 | -0.57284 | -0.21433 | 0.11489  |
| Aggressiveness           | -0.06276 | -0.26425 | 0.43907  | -0.84173 |
| Start exploration        | -0.54014 | 0.04937  | -0.12705 | -0.11142 |
| End exploration          | -0.53496 | 0.18765  | -0.12397 | -0.11724 |
| Visual exploration       | 0.06561  | 0.62784  | -0.12573 | -0.15998 |
| Exposed time             | -0.06815 | 0.11486  | 0.83459  | 0.38347  |
| First perch              | -0.52270 | 0.24295  | 0.11265  | 0.05655  |
| Boldness                 | -0.32146 | -0.31301 | 0.06873  | 0.27621  |
| Importance of components | PC1      | PC2      | PC3      | PC4      |
| SD                       | 1.7659   | 1.2412   | 1.0593   | 0.9571   |
| Proportion of variance   | 0.3898   | 0.1926   | 0.1403   | 0.1145   |
| Cumulative proportion    | 0.3898   | 0.5824   | 0.7227   | 0.8372   |

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206 **S4. Table describing observations from the VES**

207 Number of lizards of each species observed at each site. Note data obtained from  
 208 VES correspond to relative abundance estimates, but are not considered accurate  
 209 estimates of lizard densities.

| Site                       | Habitat type                      | Species                | Total                               | % total<br><i>Anolis</i> |
|----------------------------|-----------------------------------|------------------------|-------------------------------------|--------------------------|
| Markham Forest             | Natural forest                    | <i>A. sagrei</i>       | 233                                 | 92.5                     |
|                            |                                   | <i>A. distichus</i>    | 19                                  | 7.5                      |
| Fern Forest                | Natural forest                    | <i>A. sagrei</i>       | 554                                 | 99.5                     |
|                            |                                   | <i>A. carolinensis</i> | 3                                   | 0.5                      |
| Las Olas                   | Urban with <i>L. carinatus</i>    | <i>A. sagrei</i>       | 666                                 | 98.5                     |
|                            |                                   | <i>A. carolinensis</i> | 13                                  | 1.5                      |
|                            |                                   | <i>L. carinatus</i>    | 188                                 | -                        |
| Library                    | Urban with <i>L. carinatus</i>    | <i>A. sagrei</i>       | 946                                 | 99.5                     |
|                            |                                   | <i>A. carolinensis</i> | 6                                   | 0.5                      |
|                            |                                   | <i>L. carinatus</i>    | 159                                 | -                        |
| Coral Gables               | Urban without <i>L. carinatus</i> | <i>A. sagrei</i>       | 1,474                               | 93.9                     |
|                            |                                   | <i>A. carolinensis</i> | 29                                  | 1.8                      |
|                            |                                   | <i>A. distichus</i>    | 66                                  | 4.2                      |
| South Miami                | Urban without <i>L. carinatus</i> | <i>A. sagrei</i>       | 1,394                               | 84.8                     |
|                            |                                   | <i>A. carolinensis</i> | 35                                  | 2.1                      |
|                            |                                   | <i>A. distichus</i>    | 134                                 | 8.2                      |
|                            |                                   | <i>A. cristatellus</i> | 80                                  | 4.9                      |
|                            |                                   | <i>A. equestris</i>    | 1                                   | <0.01                    |
| # of lizards               |                                   | 6001                   | # of <i>L. carinatus</i> 425        |                          |
| # of <i>Anolis</i> lizards |                                   | 5576                   | Mean % of <i>A. sagrei</i> 94.8     |                          |
| # of <i>A. sagrei</i>      |                                   | 5276                   | Mean # <i>A. sagrei</i> / VES 31.35 |                          |

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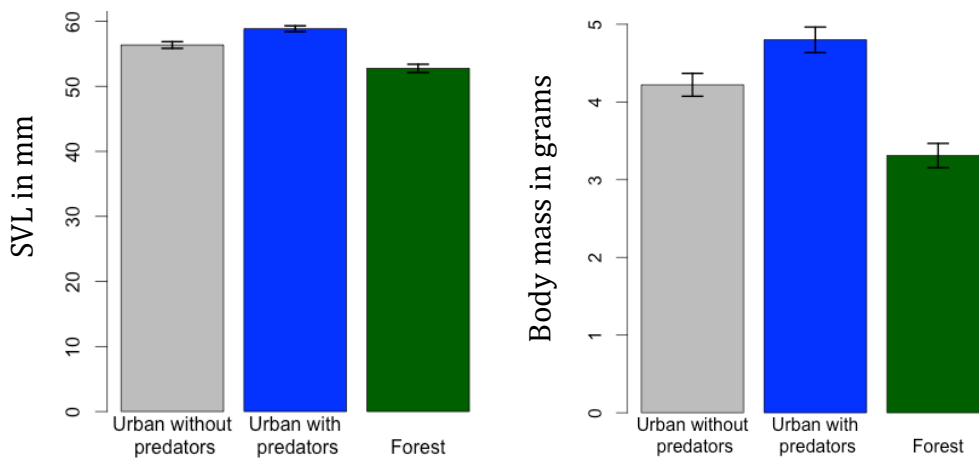
213 **S5. Body size, body condition, and behavioral traits**

214 Because behavioral traits could be affected by body size and motivational state, we  
215 investigated if morphology (SVL, body mass and body condition) played a role in  
216 determining the behaviors of *A. sagrei*. Snout-vent length (SVL in mm) was obtained  
217 by hand using a ruler and body mass (in grams) was measured with a precision  
218 digital balance. Body condition was estimated as the residuals of the correlation  
219 between log-transformed SVL and log-transformed body mass. The possible  
220 influence of these variables on behavioral traits was assessed using correlations  
221 with values obtained from the behavioral experiments as dependent variables.  
222 These correlations were estimated both by pooling all populations together and for  
223 each population separately to remove the effect of mean population differences with  
224 no causal association with the effects. In general, body mass or body condition did  
225 not affect individual-level behavioral differences.

226

227 Because we found differences in the body size and body condition of lizard  
228 populations from different habitat types (Fig S1a and S1b), we analyzed the  
229 association between body size (i.e., SVL), body condition (obtained from the log-log  
230 regression of body size in mm and body mass in grams), and the behavioral traits  
231 measured in the experiments conducted.

232



233

234 In general, no important associations between body size or body mass with the  
235 studied behavioral traits were found. Note that in some cases a correlation was  
236 found. In these cases, the table below shows that the association was the  
237 consequence of mean size and body condition differences among populations rather  
238 than these having a direct effect on behavioral traits since no effects were found  
239 when splitting these correlations among habitat types except for the number of  
240 perches used. Smaller lizards tended to be more spatially explorative in urban  
241 populations. Body condition did not have any effect on spatial exploration  
242 tendencies.  
243

|   | Body size (SVL)                         | Body condition                          |
|---|---|---|
|   | p-value of<br>Pearson's corr.<br>Coeff. | p-value of<br>Pearson's corr.<br>Coeff. |
| Exploration start time                              | 0.638                                   | 0.57                                    |
| Time to emerge after simulating predatory<br>attack | <0.005 (-0.291)                         | 0.012 (-0.248)                          |
| Urban without <i>L. carinatus</i>                   | 0.081                                   | 0.266                                   |
| Urban with <i>L. carinatus</i>                      | 0.998                                   | 0.93                                    |
| Forest  | 0.974                                   | 0.946                                   |
| Time to hide when simulating predatory attack       | 0.894                                   | 0.291                                   |
| Foraging innovation                                 | 0.23                                    | 0.489                                   |
| Escape distance                                     | 0.05 (-0.193)                           | 0.242                                   |
| Urban without <i>L. carinatus</i>                   | 0.393                                   | -                                       |
| Urban with <i>L. carinatus</i>                      | 0.971                                   | -                                       |
| Forest  | 0.143                                   | -                                       |
| Visual exploration time                             | <0.001 (0.366)                          | 0.266                                   |
| Urban without <i>L. carinatus</i>                   | 0.102                                   | -                                       |
| Urban with <i>L. carinatus</i>                      | 0.274                                   | -                                       |
| Forest  | 0.973                                   | -                                       |
| Number of perches used                              | 0.062                                   | 0.827                                   |
| Urban without <i>L. carinatus</i>                   | 0.050 (-0.506)                          | -                                       |
| Urban with <i>L. carinatus</i>                      | 0.031 (-0.415)                          | -                                       |
| Forest  | 0.342                                   | -                                       |

245 **S6. Effect sizes**

246 Urban lizards were more tolerant to human presence and reacted less aggressively  
247 when captured than forest-dwelling individuals, irrespective of their predation  
248 regime. Lizards from forests escaped from a distance that was between 78% and  
249 119% further away than urban lizard populations and number of gapes was  
250 between 48% and 54% higher. Exploratory behavior also differed between forest-  
251 and city-dwelling lizards. Urban lizards spent by mean 257% and 281% more time  
252 collecting information from the new environment before emerging from a refuge.  
253 This can have two possible explanations: either in urban lizards it is beneficial to  
254 spend more time assessing for the uncertainties associated with more complex  
255 urban habitats or forest lizards have higher assessment abilities and need less time  
256 to make an similar decisions in a novel environment. Our prediction is that forest  
257 environments are more predictable, and lizards do well without the need to spend  
258 time assessing for unknown risks while urban habitats might somehow favor more  
259 cautious behavior because animals are more frequently confronted with  
260 uncertainty. Urban lizards have become more tolerant to human presence,  
261 suggesting they do not consider humans a threat whereas when confronted with  
262 uncertainty (e.g. new experimental settings), they have a tendency to be more  
263 cautious. On the other hand, latency to emerge from refuge after the simulation of an  
264 attack was also higher in forest-dwelling lizard populations (between 76% and  
265 86%). Spatial exploration showed a different pattern in which lizards from urban  
266 populations with *L. carinatus* lizards explored less than both the urban populations  
267 without predators and forest populations (mean effects ranges from 31% and 32%).  
268 However, these differences were not significant (see Results section of the  
269 manuscript).

270

271 **S7. Effects of the presence of *L. carinatus* on foraging behavior of *A. sagrei***

272 We compared two types of effects. First, ‘instantaneous effects,’ that is whether the  
273 presence of a predator within a radius of 10 m from an *A. sagrei* lizards’ perch  
274 increased their latency to feed or decreased their time on the ground. Second, we  
275 evaluated ‘long-term effects,’ differences between sites with and without predators  
276 irrespective of the presence of a predator around the area at the time of the  
277 experimental assay in the field.

278

279 *a) Latency to feed:* Lizards spent more time before feeding when there was a visible  
280 *L. carinatus* predator at a distance of less than 10 m from its perch (right vs. middle  
281 bars) (Wilcoxon rank test:  $W = 237.5$ ,  $p\text{-value} < 0.01$ ). Interestingly, however, there  
282 was no significant difference in the latency to feed between anoles from the sites  
283 without predators (right bar) compared to those from the sites with predators when  
284 there was no *L. carinatus* visible (middle bar) (Wilcoxon rank test:  $W = 940$ ,  $p\text{-value}$   
285  $= 0.43$ ).

286

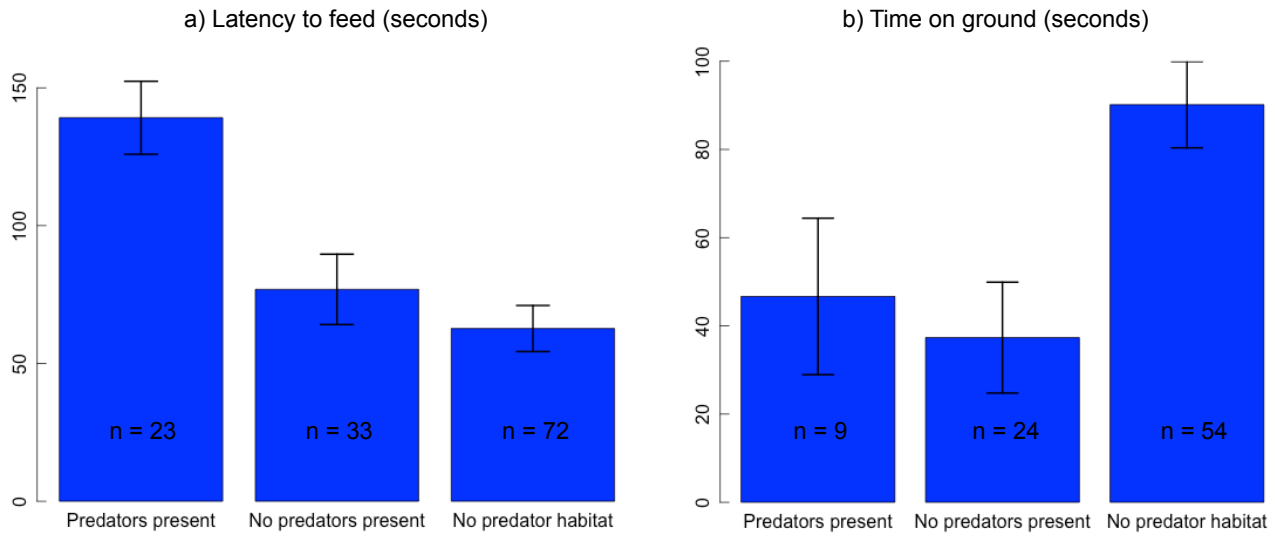
287 *b) Time spent on ground after capturing prey:* In contrast to what happens for  
288 latency to feed, lizards did not spend less time on the ground after capturing a prey  
289 when there was a predator within 10 m (right vs. middle bars) (Wilcoxon rank test:  
290  $W = 426.5$ ,  $p\text{-value} = 0.29$ ). Interestingly, however, there was a significant difference  
291 in the time spent on the ground between anoles from the sites without *L. carinatus*  
292 (right bar) compared to those from the sites with *L. carinatus* when there was no  
293 predator visible (middle bar) (Wilcoxon rank test:  $W = 939$ ,  $p\text{-value} < 0.01$ ).

294

295 Altogether, this shows that the presence of *L. carinatus* predators has an  
296 ‘instantaneous effect’ on the latency to feed in our feeding assays, but there does not  
297 seem to be more ‘long-term, permanent effects’ associated with the general  
298 presence of predators in the area were found. This suggests that decisions to  
299 descend from a perch to forage in this case are largely plastic. In contrast, there was  
300 no ‘instantaneous effect’ of the presence of *L. carinatus* predators to the time *A.*  
301 *sagrei* spent on the ground. Remarkably, there was a ‘long-term, permanent effect’



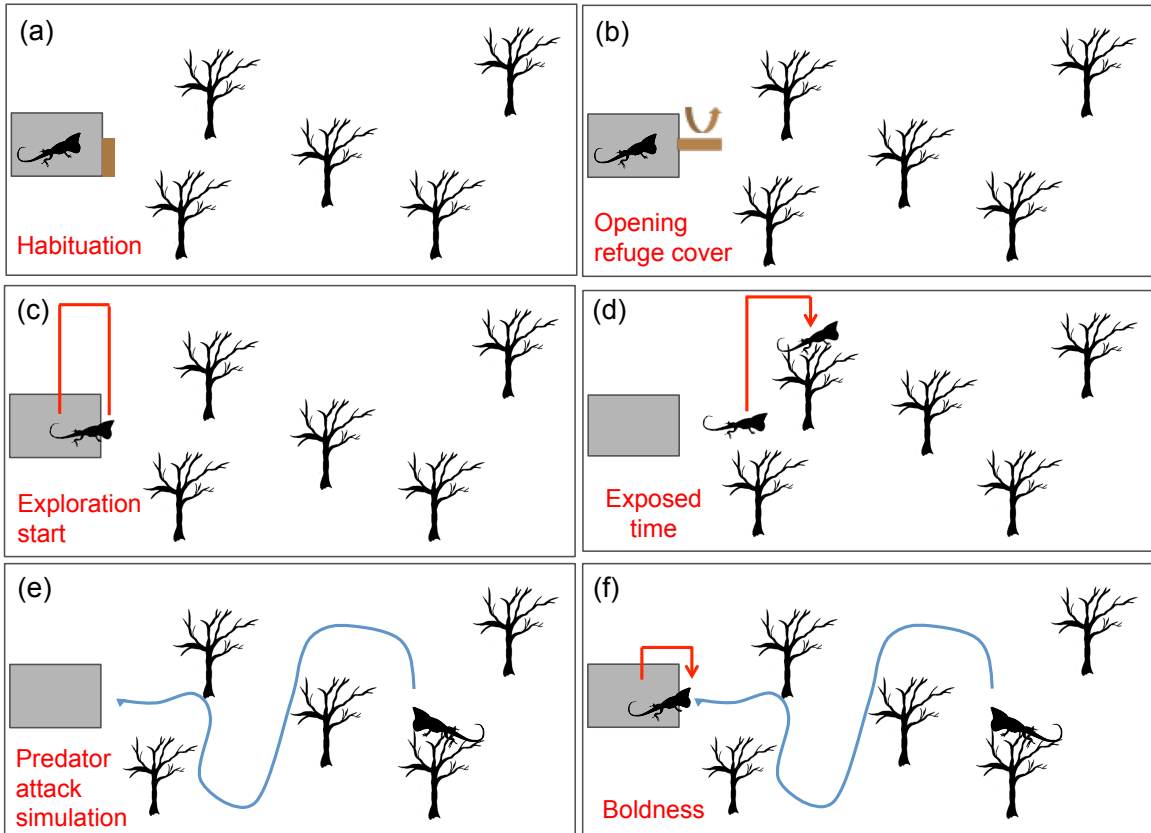
302 such that lizards from sites with *L. carinatus* spent less time on the ground  
303 irrespective of whether there were predators nearby.  
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**S8. Cartoon representation of the experimental setup described in the Methods section.**



310