

1 **Ingrained city rhythms: flexible activity timing but more persistent circadian pace in urban birds**

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11

12 **Abstract**

13 Urbanization dramatically increases the amount of light at night which may disrupt avian circadian
14 organization. We measured activity patterns of great tits breeding in the city and forest, and
15 subsequently measured two clock properties of these birds under controlled conditions: tau
16 (endogenous circadian clock speed) and after-effects (history-dependency of the clock relative to
17 previous conditions). City and forest birds showed a high repeatability of activity onset (0.60 and 0.41,
18 respectively), with no difference between habitats after controlling for date effects. Activity duration
19 and offset showed more variance, without a difference between birds from the two habitats. Tau did
20 not differ between city and forest birds, however, city birds showed stronger after-effects, taking
21 more days to revert to their endogenous circadian period. Finally, onset of activity was correlated with
22 clocks speed in both habitats. Our results suggest that potential differences in activity timing of city
23 birds is not caused by different clock speeds, but by a direct response to light. Persistence in after-
24 effects suggests a reduced sensitivity of the clock to light at night. Urbanization may select for clock
25 properties that increase the inertia of the endogenous circadian system to improve accuracy of activity
26 rhythms when exposed to noisier lighting cues.

27

28 **Keywords:** great tit, incubation, urbanization, ALAN, after-effects of entrainment, free-running period

29 **Introduction**

30 Urbanization is one of the most expressive global anthropogenic impacts. The United Nations
31 estimates that, by 2050, two-thirds of all civilians will live in cities or urban centres, urging a
32 sustainable urban planning (United Nations, 2018). The process of urbanization is known to cause
33 dramatic changes in the environment and to put organisms under contrastingly selective pressures
34 compared to forests (Johnson & Munshi-South, 2017). An obvious property of the urban environment
35 is the amount of artificial light at night (ALAN). While the increased use of ALAN is related to an
36 improvement in comfort and additional active hours (Gaston et al., 2015), it may also be disruptive to
37 the circadian system (Davies & Smyth, 2018; Dominoni et al., 2016; Kantermann & Roenneberg, 2009;
38 Russart & Nelson, 2018). This is because the light-dark cycle is the most important cue to synchronize
39 (entrain) the circadian clock (Pittendrigh, 1981; Pittendrigh & Daan, 1976b). Studies show that animals
40 modify their activity patterns in the presence of ALAN. For example, under ALAN many organisms,
41 consistently advance their daily onset, and delay their termination of activity, but ALAN has variable
42 effects on the duration of activity (Sanders et al., 2021). Diurnal birds are known to extend their
43 activities into the night in the presence of ALAN (Byrkjedal et al., 2012; Russ et al., 2015; Spoelstra et
44 al., 2018), while nocturnal organisms such as bats may emerge later and thereby reduce activity
45 duration (Boldogh et al., 2007).

46 While this change in activity patterns is clear with examples from various taxa (see Sanders et al., 2021
47 for a meta-analysis), the mechanism behind such changes is less obvious. For example, the oscillation
48 of core circadian clock components can be disrupted (Dominoni et al., 2022) or not (Alaasam et al.,
49 2021), and changes in activity patterns may not be caused by phase changes in the central circadian
50 pacemaker (Spoelstra et al., 2018). Many studies on the disruption of circadian clocks by light are done
51 in captivity with often relatively high light intensities from which animals cannot hide (e.g. Fonken &
52 Nelson, 2014 for a review, even dim light levels are often 5 lux or higher). Free-living wild animals are
53 usually exposed to much lower intensities at night, even in the urban environment where animals
54 would normally be exposed to intensities below 1 lux on average even though the light at night sources
55 may reach about 6 lux (Alaasam et al., 2021; Dominoni et al., 2014; Ouyang et al., 2017). While it is
56 known that even low light intensities are capable of disrupting the circadian system (Russart & Nelson,
57 2018), animals could simply respond with direct changes in behaviour to the presence of ALAN. For
58 example, by extending (or shortening) their activity patterns without necessarily changing their clocks
59 (Spoelstra et al., 2018). In chronobiology, this is defined as masking (Marques & Waterhouse, 1994;
60 Mrosovsky, 1999; Mrosovsky & Hattar, 2005). For example, forest birds are arguably more constrained
61 in available foraging time because the light and dark transition is much more abrupt in forests

62 compared to cities. Thus, the presence of light at night could allow birds to extend their foraging hours
63 into the night (Aschoff & von Goetz, 1989).

64 On the other hand, the highly distinct lighting environment of cities with dampened cues and low
65 predictability could indeed involve a change in clock characteristics (Dominoni et al., 2013, 2014). This
66 was suggested for blackbirds (*Turdus merula*), where birds caught in cities had dampened activity
67 rhythms and faster circadian clocks in comparison to forest conspecifics, suggesting that cities select
68 or have selected for birds with faster and weaker clocks (Dominoni et al., 2013). In the laboratory,
69 birds (great tit, *Parus major*) exposed to ALAN show a clear shift in daily expression of a core clock
70 gene (*Bmal1*) in the hypothalamus and other tissues, with some genes following, and other genes
71 retaining their normal (non-ALAN-exposed) daily phase in their expression (Dominoni et al., 2022).

72 Apart from the clock speed, biological clocks are also known to be affected by the previous conditions
73 that the organism was exposed to, affecting the period and phase of the first cycles of activity/rest in
74 constant conditions. This property is named after-effects (first described by Pittendrigh, 1960). Those
75 first cycles are interesting because they not only reflect the period and phase of previous entrainment
76 but also are indicative of initial clock inertia - with longer after-effects suggesting a lower sensitivity
77 to light (Aschoff, 1960; Oda et al., 2000; Shimomura & Menaker, 1994, Tomotani & Spoelstra, *in prep*).

78 Investigating whether the biological clocks of animals living in cities differ from forest animals is
79 challenging because it requires keeping and measuring animals in constant conditions. The few studies
80 that successfully achieved this in wild birds show contrasting results (Dominoni et al., 2013; Renthlei
81 & Trivedi, 2019), suggesting species-specific responses, or different outcomes by taking
82 measurements at moments that were not necessarily comparable between the studies. In addition to
83 the measurement of clock speed, other clock properties such as after-effects are also relevant as they
84 could reflect the sensitivity and stability of the system. It can be argued that a more stable clock would
85 be particularly important in city environments given the potentially lower contrast of the light/dark
86 transition due to the presence of ALAN that makes the light cues noisier (Beersma et al., 1999; Hut et
87 al., 1999).

88 In this study, we used wild female great tits to investigate if and how city organisms maintain the
89 accuracy of their activity rhythms despite being exposed to light at night. We first measured the
90 repeatability of activity patterns of wild city and forest great tits during incubation. Then, we measured
91 the clock properties of the same birds in constant conditions in the laboratory. We focused on two
92 aspects: the clock speed (clock period or tau) and the “history-dependence” of the clock following
93 entrainment (after-effects).

94

95 **Material and Methods**96 **a) Measurements in the field**

97 We monitored 60 nest boxes in the city of Utrecht (52° 5' 0" N, 5° 7' 0" E) and 60 in the forests of
98 Heikamp (52° 01' 50" N, 5° 50' 6" E). After great tit nest-building was detected, nests were weekly or
99 bi-weekly checked for nest-building completion, laying dates, clutch sizes and onset of incubation. We
100 then placed ThermoChron iButtons in the nest cup of 23 city and 22 forest great tits, between 7 and 5
101 days before expected chick hatching. To prevent the females from removing the devices, iButtons
102 were secured in place with a small malleable piece of wire. From the nests that we collected incubation
103 data, females were captured at the end of the breeding season and taken to the Netherlands Institute
104 of Ecology for measurements of their clocks.

105

106 **b) Measurements in the lab**

107 We measured the rhythms of 15 city and 18 forest females in constant laboratory conditions. This
108 sample differs from the field measurements as not all females could be captured. After arriving at the
109 institute, the birds were kept for one day in a cage exactly like the one in the experimental set-up but
110 exposed to the natural light/dark cycle to acclimatize. Birds were then moved from this acclimatization
111 cage to the experimental set-up in darkness at night and kept in the set-up for 14 days.

112 The experimental set-up for measuring free-running rhythms consisted of individual cages equipped
113 with doors that isolated the birds from external cues and from each other. Each cage was individually
114 equipped with a night lamp that provided dim light continuously (LLdim, 0.5 lux at perch level). Cages
115 were also equipped with perches connected to a microswitch that measured when the animal was
116 active (software developed by T&M Automation, Leidschendam, The Netherlands). Perch-hopping
117 activity was collected every 30 seconds and then binned at 2-minute intervals, thus intensity of activity
118 varied from 0 to 4 every 2-min bin. Throughout the study, the whole room containing the isolation
119 cages was kept completely dark and white noise was played continuously in the background to cover
120 any external noise and the animal's vocalizations.

121 Throughout the study, birds were offered *ad libitum* water and food (beef heart mixture, dry bird food,
122 peanuts, sunflower seeds and live mealworms). Food was refreshed daily but at random moments of
123 the day so the birds would not be able to use the feeding time as a cue to synchronize their clock. At

124 the end of the measurements, birds were moved again to the acclimatization cage and exposed to the
125 natural light/dark cycle for 2-3 days and were then released back at the capture location.

126 All experimental procedures in the lab and the field were carried out under licenses of the Animal
127 Experimental Committee of the Royal Netherlands Academy of Sciences (KNAW, protocol NIOO 20.02
128 AVD 80100 2019 9005 / IvD 1356a).

129

130 **c) Data analysis**

131 Data analyses were performed in R (version 4.1.0) (R Core Team, 2021), and unless stated otherwise,
132 we used backwards model selection, dropping non-significant terms in each step.

133

134 *Field activity onset, offset and duration*

135 In order to estimate the daily activity patterns of females, we used measurements of incubation
136 activity. The moments when females leave their nests can be easily identified by a sharp decrease in
137 nest temperatures (spikes), with the first and last temperature drops corresponding to the first and
138 last daily nest excursion, respectively. This frames the start and termination of the bird's active phase.
139 iButton data was processed using the R package IncR (Capilla-Lasheras, 2018) to extract daily onsets
140 and offsets of activity. Data was then visually inspected and we only retained measurements when
141 visual confirmation of a true offbout (sharp spike rather than a small variation in nest temperature)
142 was possible.

143 Onsets and offsets were standardized in minutes relative to civil dawn or civil dusk, respectively, using
144 the daily civil twilight times for each location. Analyses only include data from the last five days of
145 incubation (before hatching) for which data is available for the majority of nests.

146 To compare differences in daily activity onset, offset and duration, we used mixed effect models (R
147 packages "lme4", Bates et al., 2015). We included either onset, offset or duration of activity as
148 response variables and had site (city or forest), calendar date to account for seasonal effects, the
149 interaction between site and date and relative incubation stage (in days before hatching) as fixed
150 effects. To account for the fact that we had multiple measurements of the same individual, we
151 included the individual identity as a random effect. Terms were tested using a Kenward-Roger
152 approximation for F-tests, comparing models with and without effects of interest (R function
153 "KRmodcomp" from the "pbkrtest" package, Halekoh & Højsgaard, 2023).

154

155 *Repeatability of activity patterns*

156 Repeatability is the proportion of variance occurring between versus within groups (Lessells & Boag,
157 1987) and measures the consistency of the individual behaviour (Harris et al., 2020; Naguib et al.,
158 2019; Pulido, 2007). Repeatability was calculated for the onset, termination and duration of activity
159 in the field and due to the nature of the analyses, calculations were done separately for the city and
160 the forest birds. We could also only include data of individuals with at least two measurements of
161 activity. We used the “rpt” function of the “rptR” package (Stoffel et al., 2017) including individual
162 identity and calendar date of measurement as random effects and conducted 1000 bootstraps and
163 1000 permutations. For comparing differences in city and forest repeatabilities we used the 84%
164 confidence intervals as those are equivalent to z tests at the 0.05 level (Aplin et al., 2015; Harris et al.,
165 2020; Naguib et al., 2019).

166

167 *Clock properties*

168 When organisms are transferred from synchronized to constant laboratory conditions, they may
169 gradually revert from their entrained rhythm to the endogenous free-running period. This is referred
170 as “after-effects” of entrainment and indicates a history-dependency of the clock to the previous
171 synchronized conditions (Aschoff et al., 1971). In order to measure this history dependency and the
172 free-running period (τ), we extracted the daily activity onsets of the birds in constant laboratory
173 conditions. For that, we used the Chronoshop software (written by Spoelstra) to extract daily activity
174 onsets of the birds in constant laboratory conditions. The software calculates the center of gravity per
175 cycle positioned at the mean vector angle and then estimates the activity onset by going 0.5 cycle back
176 in time and detects the moment that the momentary activity exceeds the average activity in the
177 current cycle. In all cases, we excluded the day when a bird was moved to constant conditions
178 (experimental day 1, on day 0 the animal was taken to the lab and was in the acclimatization cage)
179 and the first full day of measurement (day 2) because birds were still getting used to the setup and
180 were not very active.

181 To compare the change in daily activity onsets (reflecting both the after-effects and the free-running
182 period), we used mixed effect models with the time of the onset of activity (in minutes after midnight)
183 as the response variable and relative experimental day (trend over time), relative experimental day
184 squared (for testing non-linear changes) and site (city or forest) as explanatory variables. We also
185 included the interactions between site and relative day squared and site and relative day to test
186 whether the change over time in the onset of activity differed between sites. To account for the fact

187 that we were using multiple measurements of onset of the same individual, we included the individual
188 identity as a random effect. Finally, we also corrected for bird age (second calendar year or older), for
189 the calendar day the bird arrived in the lab, for the actual light intensity value measured in the cage
190 (min 0.3, max 0.6 lux) and also included a term containing the cage “group” which accounts for the
191 neighbour cages in the room. Once more, terms were tested using a Kenward-Roger approximation
192 for F-tests.

193 Because the analysis resulted in a significant interaction between the quadratic relative date term and
194 site, with a clear quadratic relationship between date and onset of activity for city but not forest birds,
195 we conducted a post-hoc piecewise mixed model on the city bird data to test whether two lines would
196 yield a better fit than the quadratic model and if so, identify the breaking point. Afterwards, we used
197 the linear relationships before and after the breaking point to estimate the clock period during after-
198 effects and free-running for both city and forest birds and tested how the mean period differed
199 between sites and before and after the breaking point.

200

201 *Onset of activity and endogenous period*

202 We combined the data obtained in the lab and in the field to test how the timing of daily onsets of
203 activity in the field was related to the endogenous circadian period in constant conditions both during
204 the after-effects and free-running phases estimated in the previous section. Once more, we used
205 mixed effect models with onset of activity in the field (minutes relative to civil dawn) as the response
206 variable and site, hatching date, incubation date (days before hatching), clock period during after-
207 effects and clock period during free-running as fixed effects. We also included the interactions
208 between site and clock period during after-effects and site and clock period during free-running. To
209 account for the multiple measurements of field onset of the same individual, we included the
210 individual identity as a random effect. Terms were tested using a Kenward-Roger approximation for
211 F-tests.

212

213 **Results**

214 Tables with the complete statistical results and complementary plots showing individual data can be
215 found in the supplementary material.

216 A) Activity patterns and repeatability of the activity parameters

217 When mean values of activity are compared across sites, the onset of activity was significantly earlier
218 for city birds in comparison to forest birds ($F = 7.86$, $p = 0.01$, city estimate = 61.28 ± 3.95 min, forest
219 estimate = 77.57 ± 4.26 min (minutes relative to sunrise); Fig. 1a). However, this is only the case when
220 the absolute date of measurement is not taken into account. When measurement day is included in
221 the analysis, the difference between sites disappears, with a significant effect of date of measurement,
222 without a significant interaction term (interaction: $F = 0.73$, $p = 0.39$; date effect: $F = 8.62$, $p < 0.01$;
223 site effect: $F = 3.22$, $p = 0.08$; Fig. 1d). Therefore, mean differences in onset of activity are probably
224 related to the relatively earlier timing of breeding of city birds, and, consequently, earlier
225 measurement days and for, both sites, the onset of activity gets later at the same rate relative to civil
226 dawn (estimate for date (slope) = 1.25 ± 0.42 min).

227 Birds from different sites did not differ in average offset ($F = 0.07$, $p = 0.79$, city estimate = -124.58
228 ± 6.09 min, forest estimate = -127.11 ± 6.98 min; Fig. 1b) or duration of activity ($F < 0.01$, $p = 0.98$, city
229 estimate = 769.81 ± 6.81 min, forest estimate = 770.01 ± 7.73 min; Fig. 1c) regardless of the presence
230 of the date term in the model. Moreover, there was no measurement date effect, with a similar offset
231 ($F = 1.23$, $p = 0.27$, estimate = -0.81 ± 0.72 min; Fig 1e) and duration ($F = 2.06$, $p = 0.16$, estimate = 1.22
232 ± 0.84 min; Fig. 1f) of activity throughout the season.

233 The onset of activity had high repeatability in both city ($R=0.61$) and forest ($R=0.43$) birds, while for
234 offset and duration of activity repeatability was high ($R=0.54$, $R=0.49$) in city birds and moderate
235 ($R=0.21$) and low ($R=0.05$) in forest birds, respectively (Fig. 1g). For all traits, there was an overlap
236 between the 84% CI, thus the repeatabilities were not significantly different between sites (see Aplin
237 et al., 2015; Harris et al., 2020; Naguib et al., 2019).

238

239 B) Clock properties of city and forest birds

240 Upon being placed in constant conditions, the endogenous circadian period of the activity rhythms for
241 both city and forest birds eventually deviated from 24 hours with a steady duration reflecting the pace
242 of their endogenous clock. However, city and forest birds differed in the amount of time before the
243 circadian period stabilized. This took longer in city birds as a result of stronger after-effects as these
244 birds retained a 24h or slightly longer period for more circadian cycles compared to forest birds before
245 reverting to a steady, shorter tau (interaction days² and site: $F=8.19$, $p<0.01$, Fig 2a, quadratic
246 interaction estimate = 1.17 ± 0.41 , linear interaction estimate = -30.32 ± 7.09 ; estimates are for forest
247 birds relative to city birds). The model estimates also showed an earlier onset of activity for city birds
248 in the first cycles after being placed in constant conditions (city: 106.71 ± 120.73 min from civil dawn;

249 forest 210.10 \pm 126.21 min from midnight), a pattern that was eventually reversed when forest birds
250 further drifted from 24h (Fig 2a).

251 To further explore this result, in a post-hoc test we used a piecewise mixed model analysis to assess
252 when the change in period occurs, which returned the cycle day 9. A post-hoc comparison of the slopes
253 between city and forest birds showed that from 1-9 days in DD, the two sites had a significantly
254 different period, in other words, differed in the slope of the relationship between onset of activity in
255 LL and days in LL (interaction: $F=31.08$, $p<0.01$, slope city = 1.88, slope forest = -17.30) but after 9 days,
256 the slopes of both forest and city birds became similar and thus the period of the two birds was
257 basically the same (interaction: $F=1.77$, $p=0.19$; date effect: $F=78.01$, $p<0.01$, date estimate = -13.78).
258 These results were corroborated in the comparison of the mean period of the individuals, with a
259 significant interaction between site and interval (before and after the breaking point) (interaction:
260 $F=9.60$, $p<0.01$), meaning in city birds the mean period differed between intervals, but this was not
261 the case for forest birds (estimates: city birds before: 24h 2.34min \pm 3.55, after: 23h 47.55min \pm 3.36;
262 forest birds before: 23h 42.57min \pm 3.69, after: 23h 45.52 min \pm 3.48; Fig 2b). Therefore, city and forest
263 birds do not differ in their tau, but they do differ in their after-effects of entrainment.

264

265 C) Relationship between onset of activity and clock properties

266 Neither of the interactions between site and period explained the variation in onset of activity (1-9
267 days: $F=0.05$, $p=0.82$, estimate = 0.09 \pm 0.40; 9-14 days: $F=0.02$, $p=0.88$, estimate = -0.08 \pm 0.57). The
268 onset of activity in the field was also not related to the period of activity in constant conditions when
269 measured before 9 days (during after-effects; $F=0.28$, $p=0.60$, estimate = -0.11 \pm 0.20 Fig 3a). But they
270 were significantly related when measured after 9 days (after completion of after-effects) where birds
271 with shorter tau also started their activity earlier in the field ($F=5.09$, $p=0.03$; estimate = 0.55 \pm 0.24;
272 3b). The effect of site was significant, and, similarly to the results from part A but using a smaller
273 dataset, showed an earlier onset of activity in the field for city birds ($F=5.12$, $p=0.03$, city = 37.60.23
274 \pm 16.63 min; forest = 50.17 \pm 18.15 min).

275

276 Discussion

277

278 In this study, we showed that city and forest birds while having high repeatability in their onset of
279 activity, do not differ in the timing of their activity patterns when date effects are taken into account.

280 We also showed that city birds, despite having a similar circadian period compared to forest birds, had
281 a stronger history-dependency in their circadian period to the previous condition. This is expressed by
282 a difference in retention of the ~24-hour interval between consecutive onsets of activity after taking
283 forest and city birds from the external light-dark cycle into constant (time-isolated) conditions in the
284 laboratory. There, on average, forest birds directly revert to their internal, endogenous circadian
285 period whereas city birds needed roughly nine circadian cycles to reach a circadian period of similar
286 duration.

287 A difference in onset of activity between free-ranging forest and city birds, with city birds starting their
288 activity earlier compared to forest birds, is in line with previous comparative studies between urban
289 and non-urban birds. Such differences were observed in blackbirds (*Turdus merula*, Dominoni et al.,
290 2013) an effect linked to artificial light at night. Indeed, blackbirds that are individually exposed to
291 higher artificial light levels start their activity earlier compared to birds exposed to less light (Dominoni
292 et al., 2014; Russ et al., 2015). In the laboratory, low-intensity light at night strongly advances activity
293 patterns in great tits (*Parus major*) in a dose-dependent manner (de Jong et al., 2016; Spoelstra et al.,
294 2018). Blue tits with (part of) their territory illuminated by light posts started their activity much earlier
295 compared to birds in dark (forest) territories (Kempenaers et al., 2010, but see da Silva et al., 2017).
296 However, in our case, the difference we found in daily onset of activity may well be related to the
297 earlier start of breeding in city birds, as the difference in daily onset disappeared with the inclusion of
298 date in the model. Birds breeding earlier in spring may advance foraging activity just because of the
299 relative short natural day length; a comparable effect has been shown for the dark phase in
300 experimental photoperiods for rodents in the laboratory (Refinetti, 2004). Whether the artificial light
301 at night in urban environments in fact facilitates earlier foraging by disturbing the perception of day
302 length in great tits remains to be tested.

303 One of the challenges in the field of Chronobiology is how ubiquitous clock properties govern activity
304 in the “real world”. For example, how variation in tau is connected to behavioural or physiological
305 differences (Helm et al., 2017). Here we found a correlation between tau and activity, where shorter
306 individual circadian period length is linked to an earlier onset of daily activity. This is in line with the
307 expectations set by previous studies (e.g. Aschoff & Wever, 1966; Duffy et al., 2001), although this
308 effect was not found in an earlier study done in the same species (Helm & Visser, 2010). Interestingly,
309 in our study, the outdoor daily onset of both forest and city birds could be linked to circadian period
310 as measured in the lab. In an earlier study, a comparable link was observed between circadian period
311 and daily onset in city, but not in forest blackbirds (Dominoni et al., 2013). Differences between this
312 and our study could be due to site differences (e.g variation in ALAN), species-specific clock properties,
313 ecological differences (e.g. great tits are smaller and breed in cavities) or differences in response of

314 males and females (their study only used males and we only used females). The males of our
315 incubating females could have started their activity earlier in the cities when compared to forests as
316 it is known that artificial light has a strong effect on dawn song onset in great tit males when defending
317 their territory (da Silva et al., 2015). Seasonal timing of females may in turn be affected by males via
318 their song activity, which has been suggested to have behavioural and physiological effects on female
319 breeding phenology (Bentley et al., 2000; Maney et al., 2007). Finally, tau was measured at a different
320 reproductive stage than activity (post-incubation) and seasonal effects could impact the absolute tau
321 value. Such effects, however, should not have modified the relationship between tau and activity,
322 where early birds have shorter late birds longer tau values.

323 We can only speculate why city birds show after-effects, and forest birds do not. Similarly to
324 differences in tau, it is unknown what the after-effects would mean for an organism in the wild, but
325 the ubiquity of such clock property makes such speculation interesting. On one hand, the differences
326 in after-effects could be a result of a (genetic) difference in the clock itself of city and forest birds. So-
327 called after-effects have been extensively reported at the behavioural level (Pittendrigh & Daan,
328 1976a). Likewise, after-effects in internal clock speed have been found at the cellular level (Meijer et
329 al., 2010; Refinetti, 1998; Roenneberg & Mellow, 2005), but its underlying molecular mechanism is
330 still not fully understood (but see Azzi et al., 2014). Despite that, the general idea is that after-effects
331 originate from a more stable phase-relationship between multiple putative oscillators in the circadian
332 pacemaker. These phase-relationships may be established by prior entrainment and cause the clock
333 to be more stable (Oda et al., 2000; Pittendrigh & Daan, 1976c; Shimomura & Menaker, 1994).
334 Moreover, a clock with a period closer to 24 hours is argued to be more stable and precise (Daan &
335 Beersma, 2002). From a functional prospect, such a robust circadian clock, precisely resonant with the
336 external cycle could be beneficial in city environments, as the sharp contrast between the light and
337 dark of the natural cycle is blurred by high levels of artificial light in cities at night. This is supported
338 by the relatively high repeatability values of activity patterns that we found for the city birds. Thus, a
339 circadian clock that is intrinsically more stable and less responsive to the aberrant and potentially
340 confusing light pattern in cities is potentially adaptive.

341 While not a measure of light sensitivity per se, the literature on circadian rhythms provides support
342 for the hypothesis that the difference in after-effects could indirectly represent differences in light
343 sensitivity due to a more stable clock. For example, when birds are exposed to higher (constant) light
344 intensities in the lab, the period of their circadian rhythm shortens (one of the "Aschoff rules", Aschoff,
345 1960; Aschoff & Wever, 1966). Following this logic, if the forest birds are more sensitive to light, and
346 even when exposed to only 0.5 lux in the lab, they would still experience it as brighter than city birds,
347 resulting directly in a shorter tau. City birds, less sensitive to the constant dim light conditions, would

348 only start shortening tau after a sensitization of the circadian system to light. Similarly, the lower
349 sensitivity to light of city birds is also supported by behavioural studies where forest great tits
350 responded stronger to light at night by starting onset earlier in the lab (Ulgezen et al., 2019), moreover,
351 the sensitivity of the great tit circadian system to light is similar to what has been reported to other
352 species (Tomotani & Spoelstra, in prep.). In an environment with less contrast between day and night
353 and fluctuations in ALAN, a more stable clock, less sensitive to perturbations of light could be
354 beneficial. This contrasts Dominoni et al (2013) findings where the weaker city zeitgeber is suggested
355 to select for alternative activity patterns (reduced clock robustness). Our data for city birds, where
356 individuals maintain ~24h rhythms for several cycles after being placed in constant conditions do not
357 support less robust clocks in city birds.

358 Alternatively, the disparity in after-effects between city and forest birds could originate from non-
359 clock differences, and result from a direct modulation of the circadian system in response to the light.
360 For example, via a difference in perception of day length of city and forest birds. The effect of
361 photoperiod in prior entrainment on after-effects has been experimentally shown (Pittendrigh &
362 Daan, 1976a). Here, such differences between city and forest birds may be present, and result of the
363 excessive additional artificial light in the dark phase of the urban cycle. A difference in perception of
364 day length is plausible as it may cause city birds to breed earlier in the season as we observed. This is
365 in line with birds breeding earlier in response to controlled experimental light in forests (Dominoni et
366 al., 2020). It is also possible that there is a difference in sensitivity to light between city and forest
367 birds as reported above, but this difference originate from effects on the input pathway to the clock,
368 rather than on the clock itself.

369 A yet separate explanation could be related to stress coping mechanisms. Stressful conditions of cities
370 could have selected mechanisms that allow birds to live in such environments (Isaksson, 2015) and in
371 turn have effects on the rhythms measured in lab (in isolation). In earlier studies, a possible link
372 between rhythmicity and response to social isolation (stress) has been shown in quail (Formanek et
373 al., 2011). Since we measured birds solitary in cages for obtaining the clock properties measurements,
374 we cannot fully rule out a potential effect of stress on the circadian organization, although great tits
375 live in a different social structure compared to quail.

376 From a broader perspective, our study highlights the potential of comparing city and forest organisms
377 to study how biological clocks may evolve in response to urban environments. An important,
378 remaining question is whether the differences in the circadian properties between city and forest birds
379 observed here are caused by local adaptation of clocks, or are merely a phenotypic response. There is
380 evidence that genetic change can be rapid in cities (Caizergues et al., 2021; Harris et al., 2013; Hendry

381 et al., 2017), and the phenotypic differences between city and forest birds could indeed reflect genetic
382 differences in the response of their clocks to light. However, the high plastic nature of activity patterns
383 and the reversible nature of clock properties (e.g. after-effects can be easily induced by differences in
384 the perceived photoperiod) (Azzi et al., 2014; Pittendrigh & Daan, 1976a), suggest a stronger role of
385 plasticity rather than genetic change. Such plasticity would indicate the ability of the circadian system
386 to cope with rapid alterations in the light environment caused by urbanization. This would be
387 important in cities as a circadian system that continues to run with a period of 24 hours would retain
388 the highest accuracy even when exposed to noisy urban cues (Daan & Beersma, 2002), allowing
389 animals to keep track of time. This in turn could lead to higher survival as animals would better predict
390 challenging moments of the day/night cycle (DeCoursey et al., 2000; Spoelstra et al., 2016). The
391 separation between phenotypic plasticity and genetic changes is however not trivial and requires
392 specific experimental approaches in future studies.

393

394 **Data accessibility**

395 All data supporting this manuscript is available at:

396 <https://dataverse.nl/dataset.xhtml?persistentId=doi:10.34894/8KYFYO>

397

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409

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613 **Figure captions**

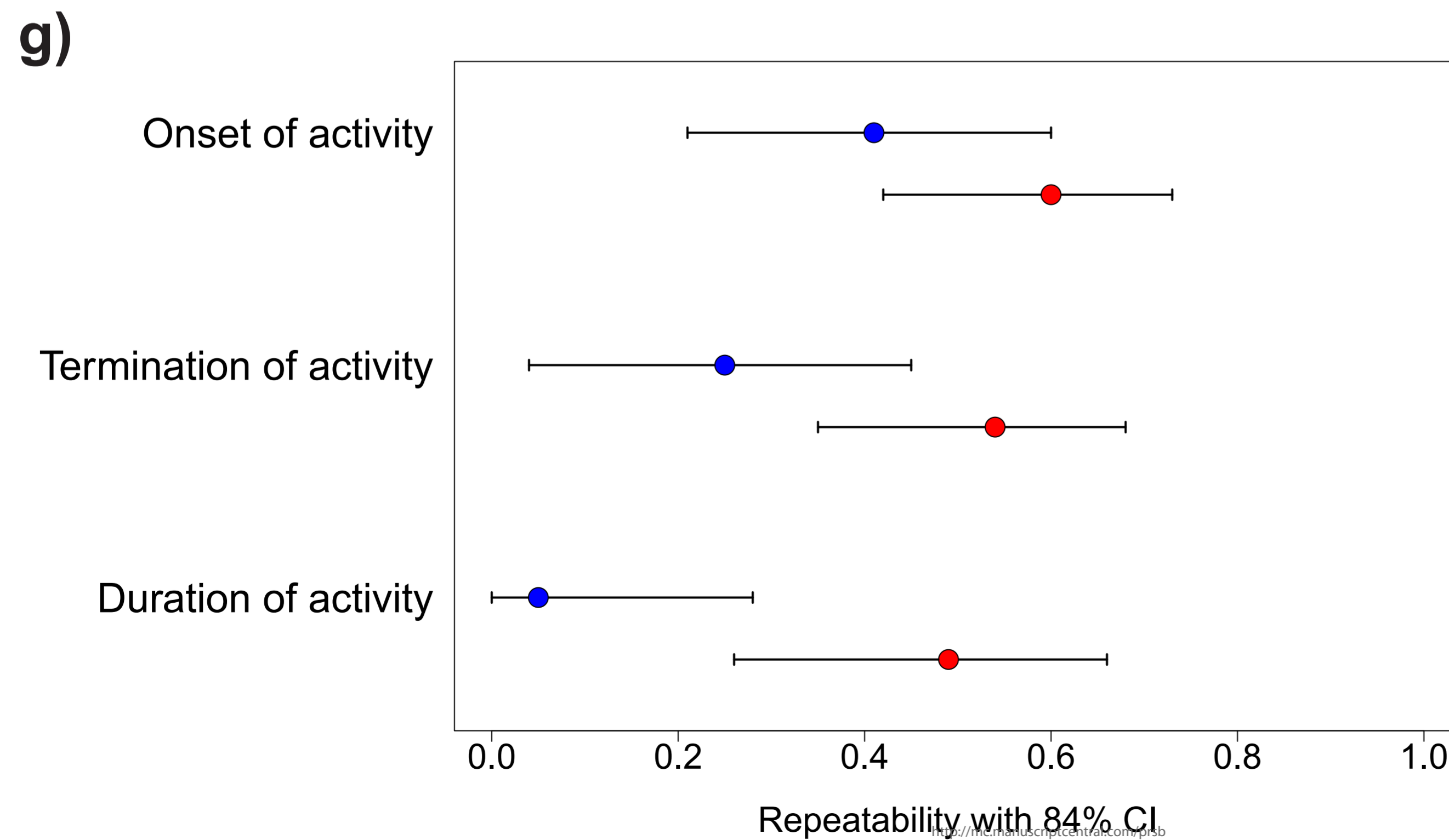
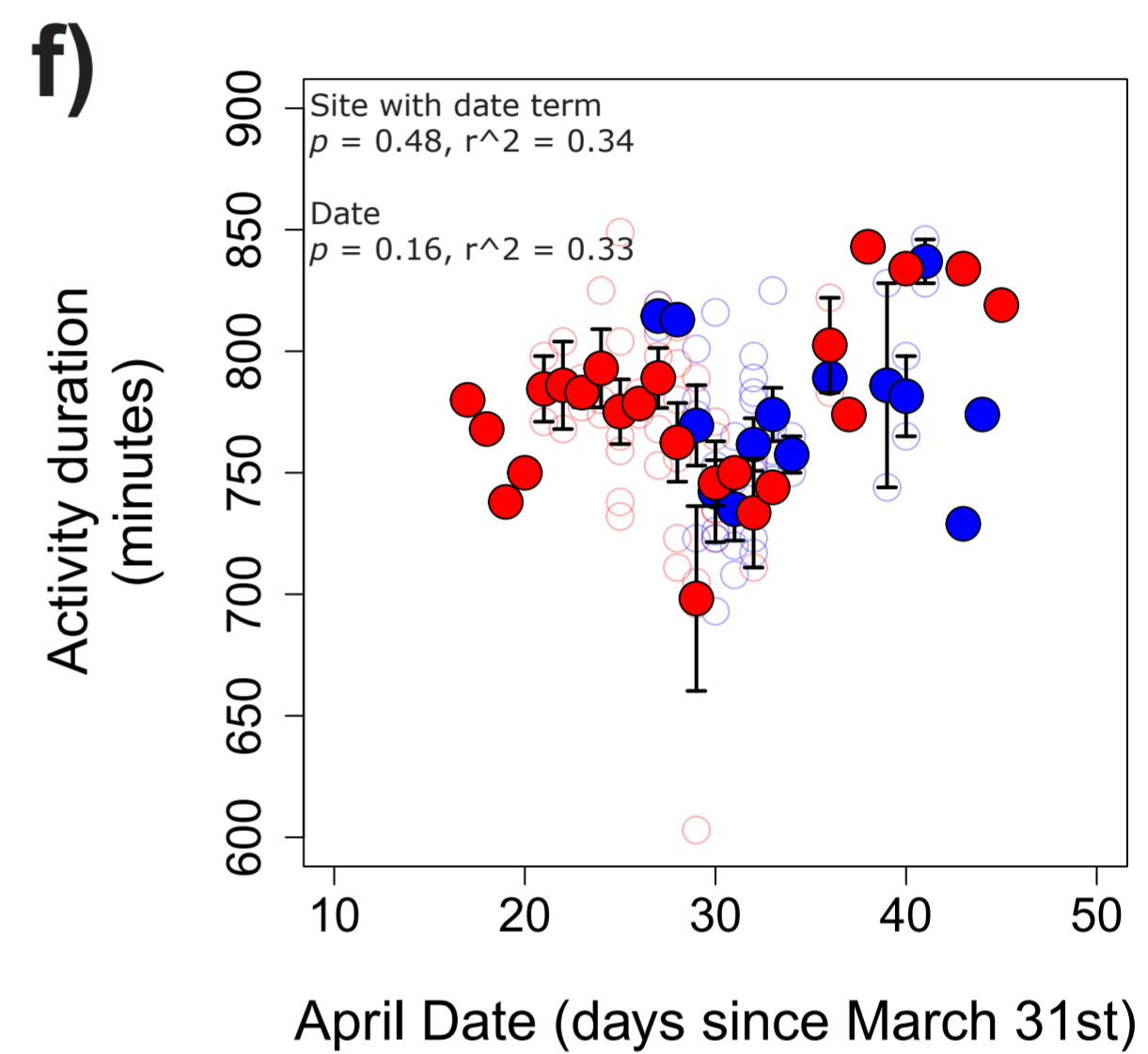
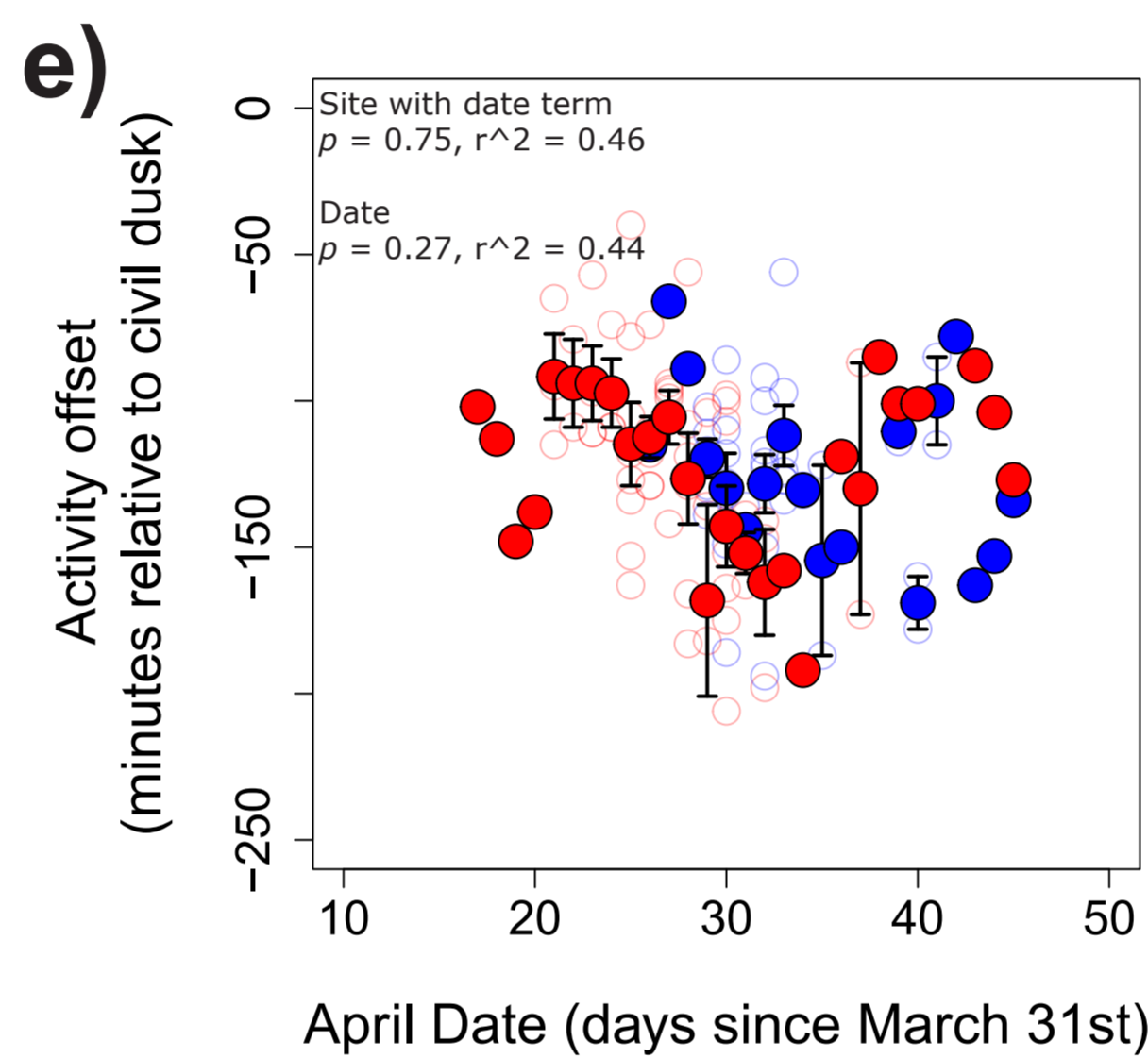
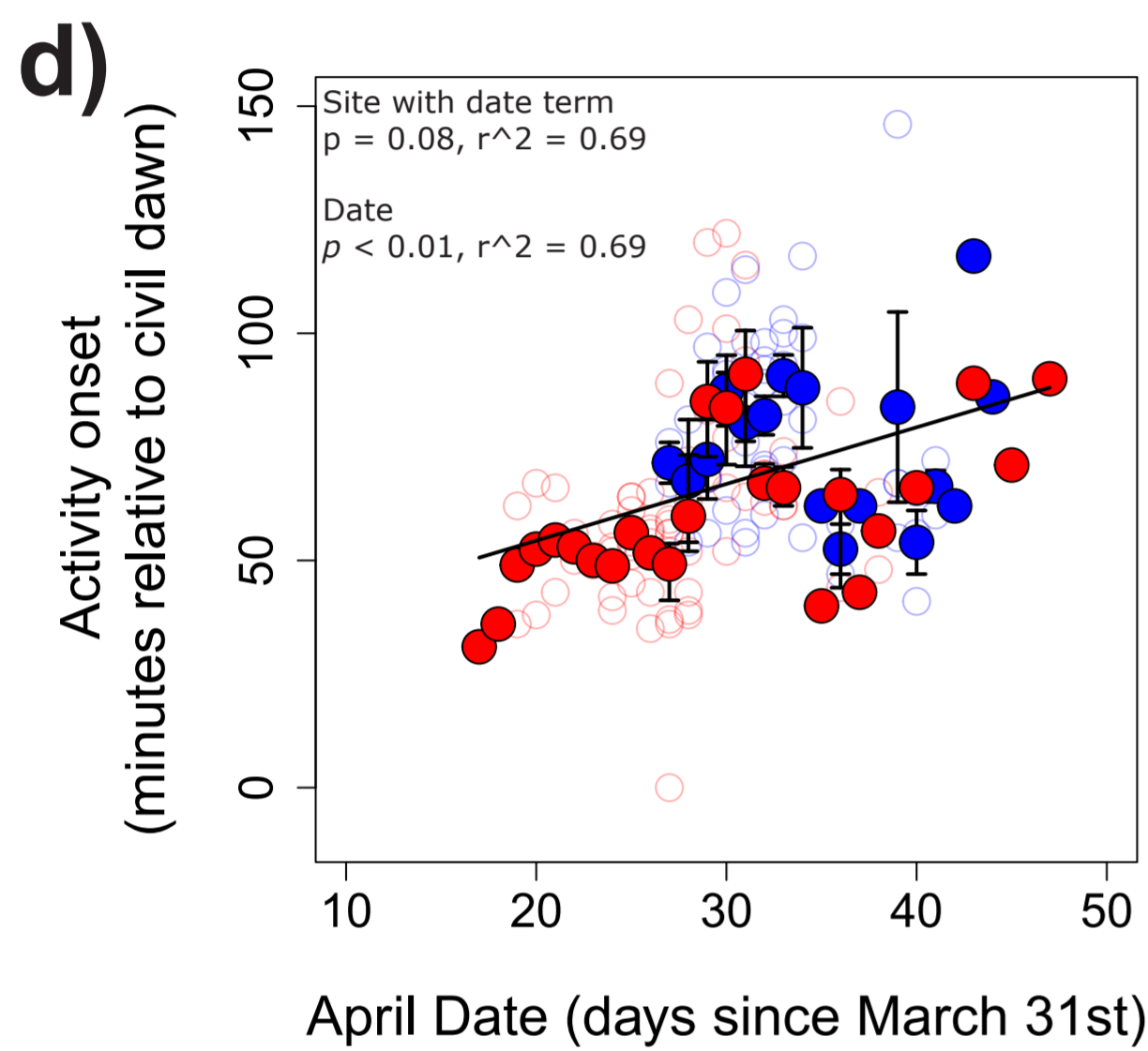
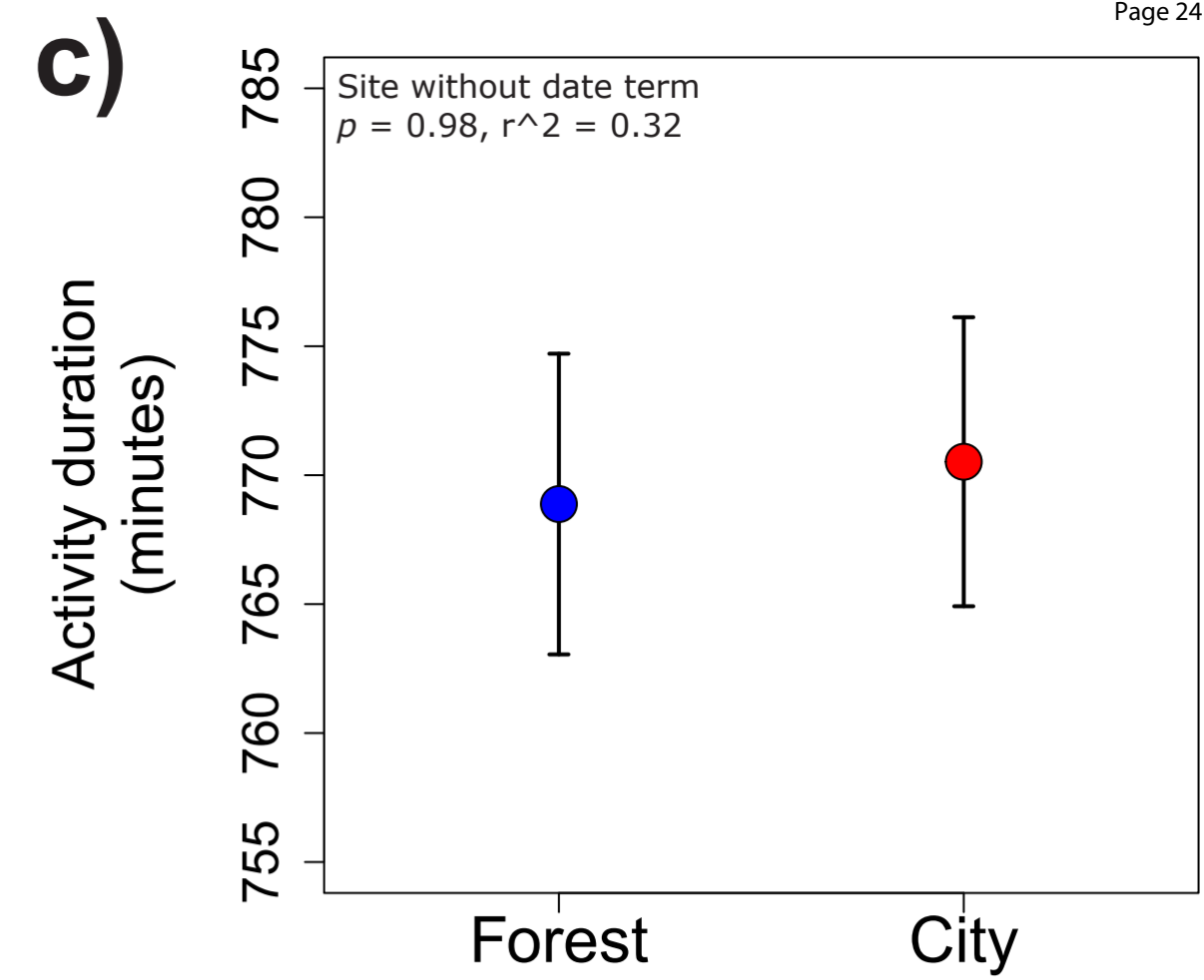
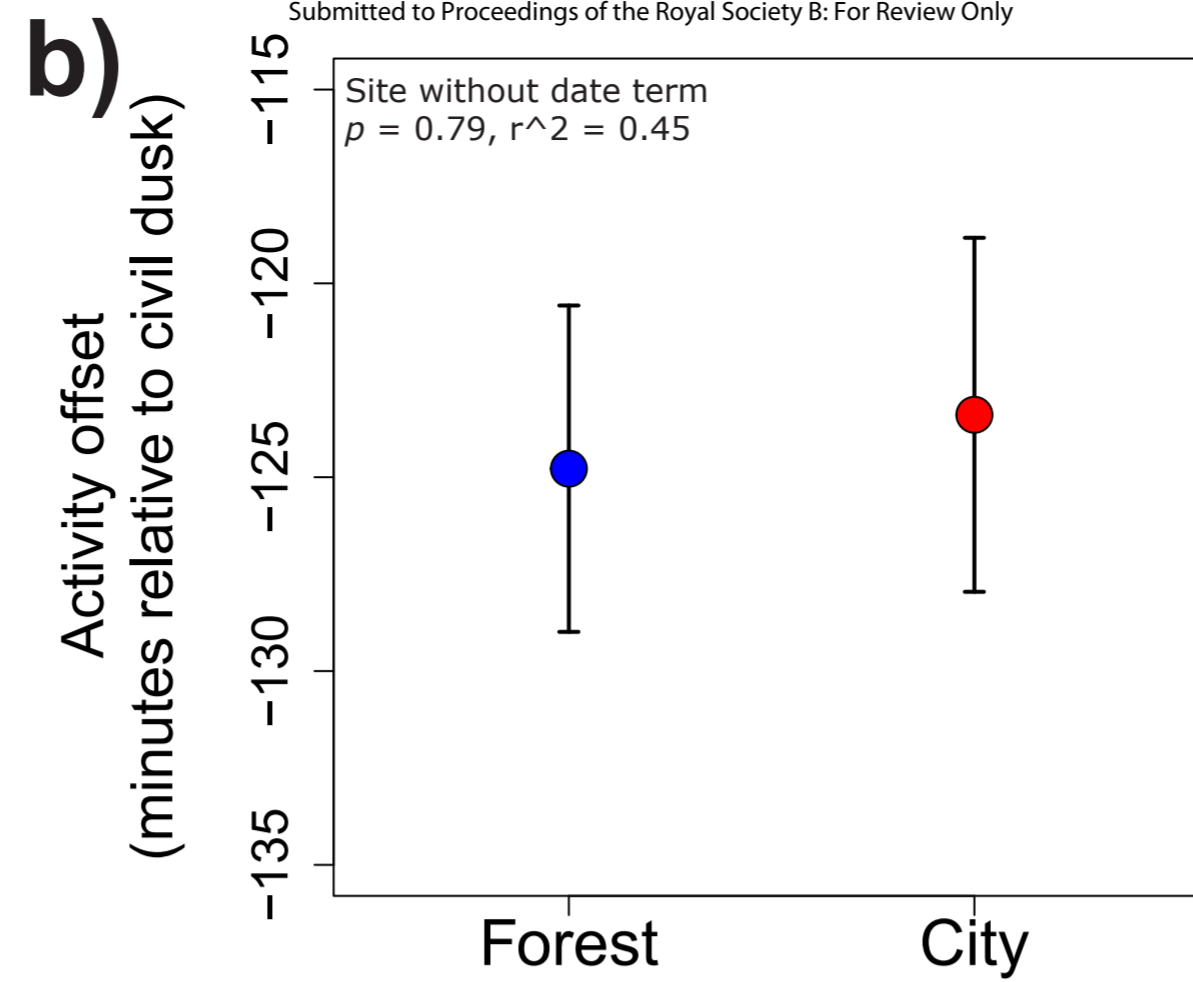
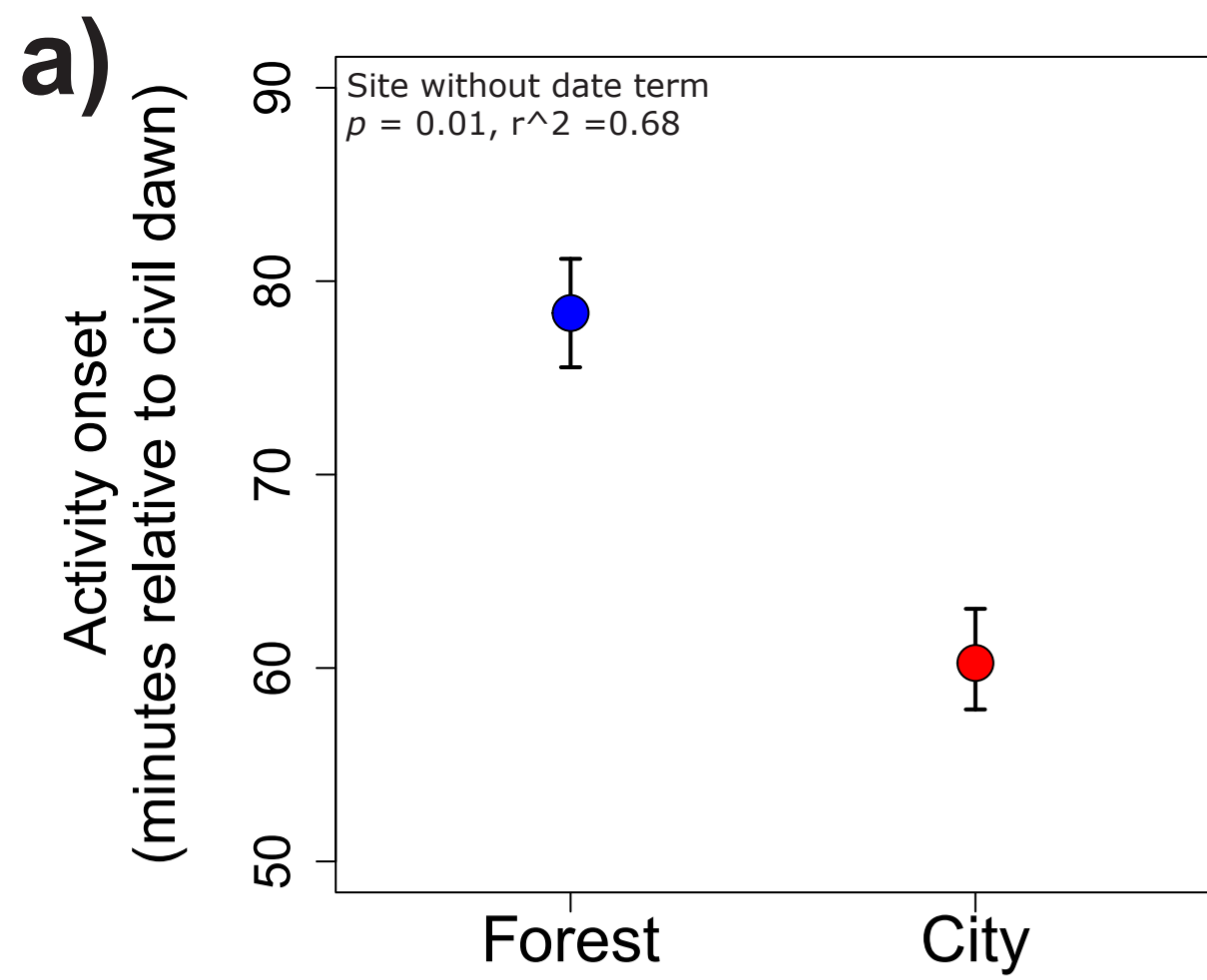
614 **Figure 1: Activity patterns and repeatability of activity during incubation in city and forest birds.** Raw
615 mean values and standard errors per site for **a)** activity onset **b)** activity offset and **c)** activity duration.
616 Change over the season in **d)** activity onset **e)** activity offset and **f)** activity duration. **g)** Repeatability
617 with 84% confidence intervals of the onset and termination of activity. Onsets and offsets of activity
618 are calculated relative to civil dawn and dusk, respectively. Red symbols = city birds, blue symbols =
619 forest birds, closed symbols = means and standard errors, open symbols = raw measurements, lines =
620 model predictions.

621

622 **Figure 2: Clock properties of city and forest birds. a)** Change in activity onset (in minutes relative to
623 civil dawn) of birds kept in constant conditions in the lab (DD). **b)** Means and standard errors of the
624 period length of urban and forest birds measured between 1 and 9 days and between 9 and 14 days
625 in DD. Values are plotted as average change in minutes per day of the onset of activity. Red symbols
626 = city birds, blue symbols = forest birds, closed symbols = means and standard errors, open symbols =
627 raw measurements, lines = model predictions.

628

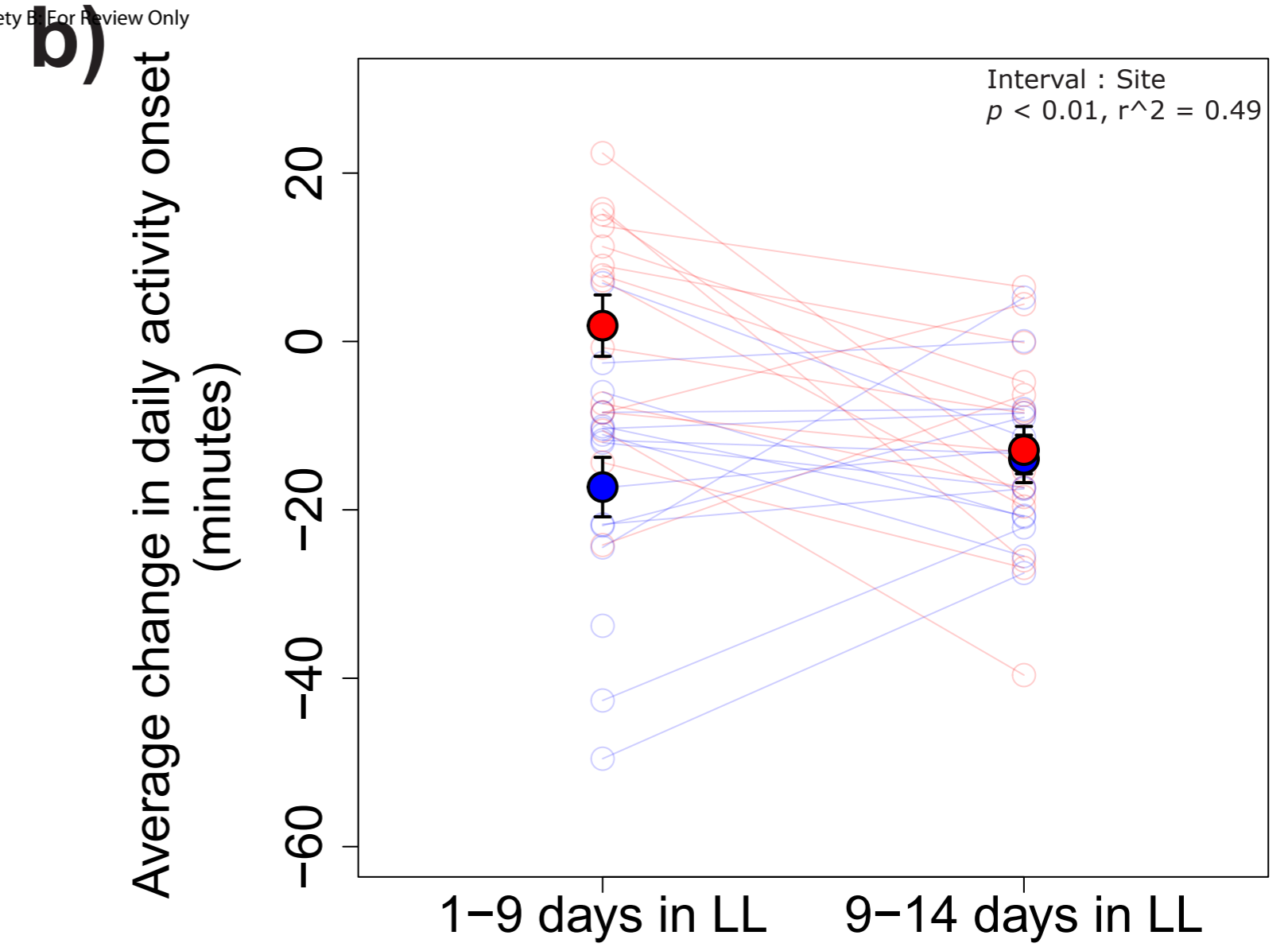
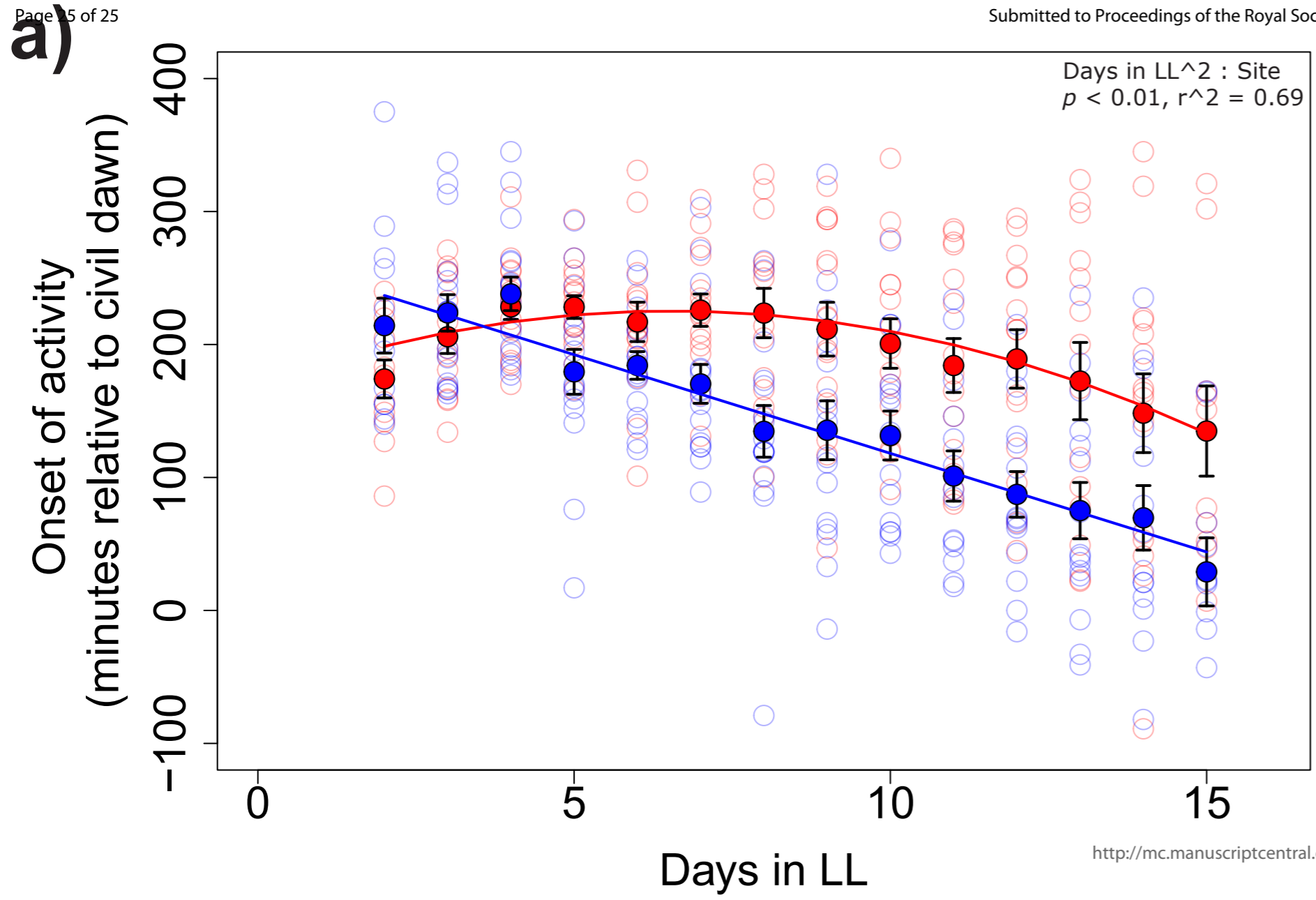
629 **Figure 3: Relationship between onset of activity in the field and clock properties. a)** Activity onset
630 relative to period length measured from 1 to 9 days in constant conditions in the lab (DD). **B)** Activity
631 onset relative to the period length measured from 9 to 14 days in constant conditions in the lab (DD).
632 Activity onset is given as minutes relative to civil dawn, period length is given as change in minutes in
633 daily activity onset in minutes relative to 24 hours.

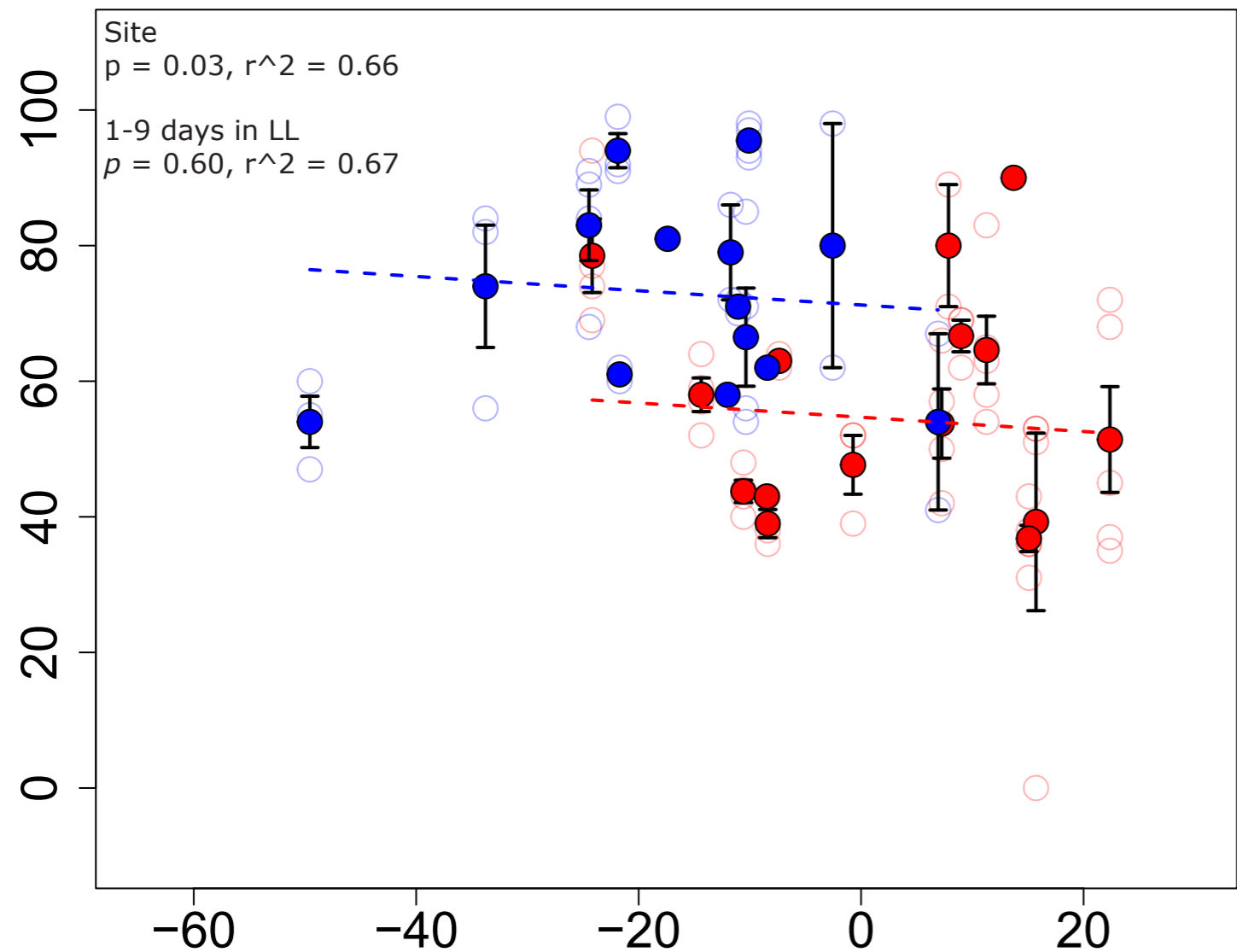
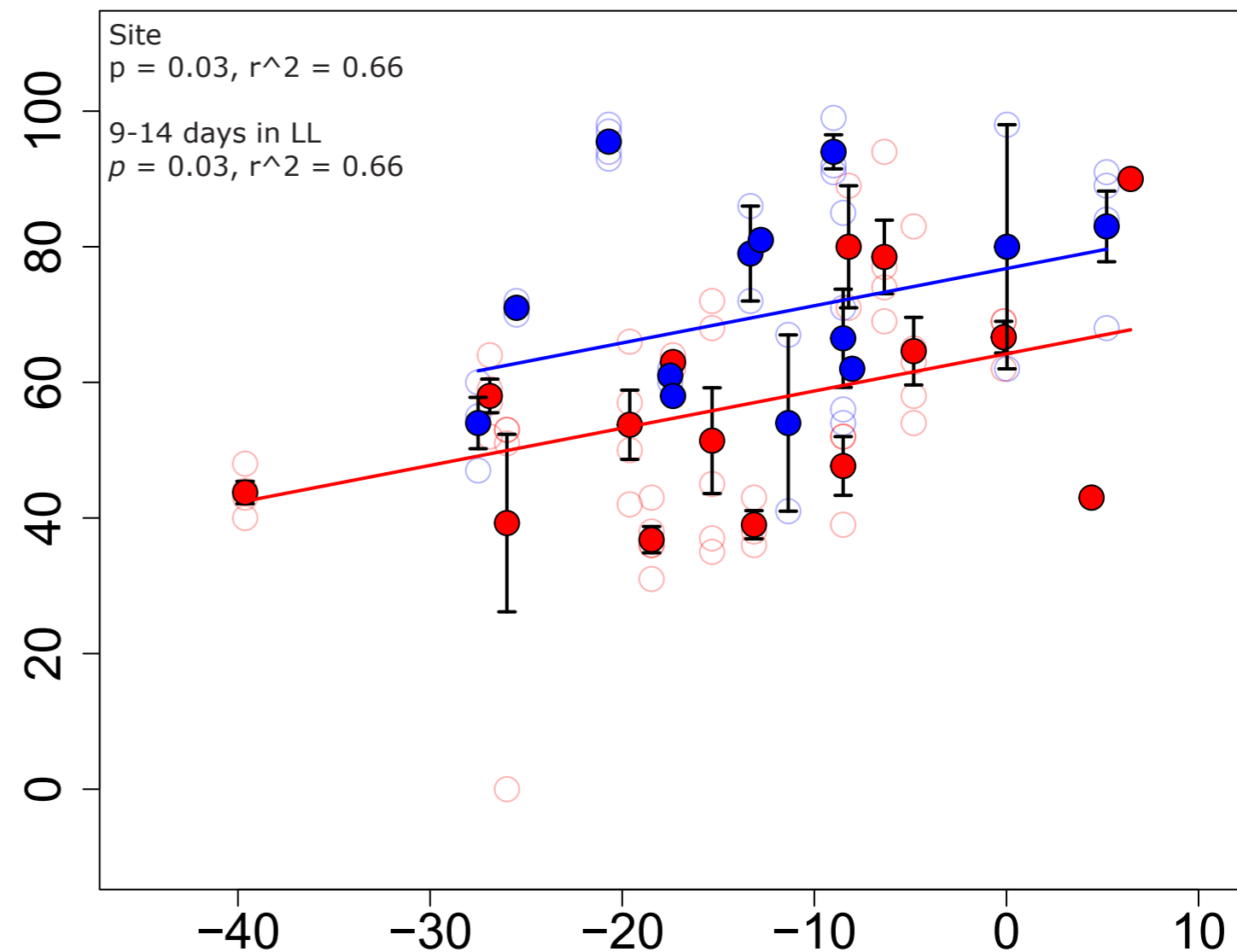


Forest birds



City birds



a)Onset of field activity
(minutes relative to civil dawn)1-9 days in LL - average change in daily activity onset
(minutes)**b)**Onset of field activity
(minutes relative to civil dawn)9-14 days in LL - average change in daily activity onset
(minutes)