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

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

1. Introduction

Urbanization is one of the most expressive global anthropogenic impacts. The United Nations estimates that, by 2050, two-thirds of all civilians will live in cities or urban centres, urging a sustainable urban planning [1]. The process of urbanization is known to cause dramatic changes in the environment and to put organisms under contrastingly selective pressures compared with forests [2]. An obvious property of the urban environment is the amount of artificial light at night (ALAN). While the increased use of ALAN is related to an improvement in comfort and additional active hours [3], it may also be disruptive to the circadian system [4–7]. This is because the light–dark cycle is the most important cue to synchronize (entrain) the circadian clock [8,9]. Studies show that animals modify their activity patterns in the presence of ALAN. For example, under ALAN, many organisms consistently advance their daily onset and delay their termination of activity, but ALAN has variable effects on the duration of activity [10]. Diurnal birds are known to extend their activities into the night in the presence of ALAN [11–13], while nocturnal organisms such as bats may emerge later and thereby reduce activity duration [14].

While this change in activity patterns is clear, with examples from various taxa (see [10] for a meta-analysis), the mechanism behind such changes is less obvious. For example, the oscillation of core circadian clock components can be disrupted [15] or not [16], and changes in activity patterns may not be caused by phase changes in the central circadian pacemaker [13]. Many studies on the disruption of circadian clocks by light are done in captivity with often relatively high light intensities from which animals cannot hide (e.g. [17] for a review, even dim light levels are often 5 lux or higher). Free-living wild animals are usually exposed to much lower intensities at night, even in the urban environment where animals would normally be exposed to intensities below 1 lux on average even though the light at night sources may reach about 6 lux [16,18,19]. While it is known that even low light intensities are capable of disrupting the circadian system [7], animals could simply respond with direct changes in behaviour to the presence of ALAN. For example, by extending (or shortening) their activity patterns without necessarily changing their clocks [13]. In chronobiology, this is defined as masking [20–23]. For example, forest birds are arguably more constrained in available foraging time because the light and dark transition is much more abrupt in forests compared with cities. Thus, the presence of light at night could allow birds to extend their foraging hours into the night [24].

On the other hand, the highly distinct lighting environment of cities with dampened cues and low predictability could indeed involve a change in clock characteristics [18,24]. This was suggested for blackbirds (*Turdus merula*), where birds caught in cities had dampened activity rhythms and faster circadian clocks in comparison with forest conspecifics, suggesting that cities select or have selected for birds with faster and weaker clocks [24]. In the laboratory, birds (great tit, *Parus major*) exposed to ALAN show a clear shift in daily expression of a core clock gene (Bmal1) in the hypothalamus and other tissues, with some genes following, and other genes retaining their normal (non-ALAN-exposed) daily phase in their expression [15].

Apart from the clock speed, biological clocks are also known to be affected by the previous conditions that the organism was exposed to, affecting the period and phase of the first cycles of activity/rest in constant conditions. This property is named after-effects (first described by [25]). Those first cycles are interesting because they not only reflect the period and phase of previous entrainment but also are indicative of initial clock inertia—with longer after-effects suggesting a lower sensitivity to light [26–28].

Investigating whether the biological clocks of animals living in cities differ from forest animals is challenging because it requires keeping and measuring animals in constant conditions. The few studies that successfully achieved this in wild birds show contrasting results [24,29], suggesting species-specific responses, or different outcomes by taking measurements at moments that were not necessarily comparable between the studies. In addition to the measurement of clock speed, other clock properties such as after-effects are also relevant as they could reflect the sensitivity and stability of the system. It can be argued that a more stable clock would be particularly important in city environments given the potentially lower contrast of the light/dark transition due to the presence of ALAN that makes the light cues noisier [30,31].

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

2. Material and methods

(a) Measurements in the field

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

(b) Measurements in the laboratory

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

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

Throughout the study, birds were offered ad libitum water and food (beef heart mixture, dry bird food, peanuts, sunflower seeds and live mealworms). Food was refreshed daily but at random moments of the day so the birds would not be able to use the feeding time as a cue to synchronize their clock. At the end of the measurements, birds were moved again to the acclimatization cage and exposed to the natural light/dark cycle for 2–3 days and were then released back at the capture location.

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

(c) Data analysis

Data analyses were performed in R (v. 4.1.0) [32], and unless stated otherwise, we used backwards model selection, dropping non-significant terms in each step.

(i) Field activity onset, offset and duration

In order to estimate the daily activity patterns of females, we used measurements of incubation activity. The moments when females leave their nests can be easily identified by a sharp decrease in nest temperatures (spikes), with the first and last temperature drops corresponding to the first and last daily nest excursion, respectively. This frames the start and termination of the bird's active phase. iButton data was processed using the R package IncR [33] to extract daily onsets and offsets of activity.

Data were then visually inspected and we only retained measurements when visual confirmation of a true off-bout (sharp spike rather than a small variation in nest temperature) was possible.

Onsets and offsets were standardized in minutes relative to civil dawn or civil dusk, respectively, using the daily civil twilight times for each location. Analyses only include data from the last 5 days of incubation (before hatching) for which data is available for the majority of nests.

To compare differences in daily activity onset, offset and duration, we used mixed-effect models (R packages 'lme4', [34]). We included either onset, offset or duration of activity as response variables and had site (city or forest), calendar date to account for seasonal effects, the interaction between site and date and relative incubation stage (in days before hatching) as fixed effects. To account for the fact that we had multiple measurements of the same individual, we included the individual identity as a random effect. Terms were tested using a Kenward–Roger approximation for *F*-tests, comparing models with and without effects of interest (R function 'KRmodcomp' from the 'pbkrtest' package, [35]).

(ii) Repeatability of activity patterns

Repeatability is the proportion of variance occurring between versus within groups [36] and measures the consistency of the individual behaviour [37–39]. Repeatability was calculated for the onset, termination and duration of activity in the field and due to the nature of the analyses, calculations were done separately for the city and the forest birds. We could also only include data of individuals with at least two measurements of activity. We used the 'rpt' function of the 'rptR' package [40] including individual identity and calendar date of measurement as random effects and conducted 1000 bootstraps and 1000 permutations. For comparing differences in city and forest repeatabilities we used the 84% confidence intervals as those are equivalent to z tests at the 0.05 level [37,38,41].

(iii) Clock properties

When organisms are transferred from synchronized to constant laboratory conditions, they may gradually revert from their entrained rhythm to the endogenous free-running period. This is referred as 'after-effects' of entrainment and indicates a history dependency of the clock to the previous synchronized conditions [42]. In order to measure this history dependency and the free-running period (tau), we extracted the daily activity onsets of the birds in constant laboratory conditions. For that, we used the Chronoshop software (written by K. Spoelstra) to extract daily activity onsets of the birds in constant laboratory conditions. The software calculates the centre of gravity per cycle positioned at the mean vector angle and then estimates the activity onset by going 0.5 cycle back in time and detects the moment that the momentary activity exceeds the average activity in the current cycle. In all cases, we excluded the day when a bird was moved to constant conditions (experimental day 1, on day 0 the animal was taken to the laboratory and was in the acclimatization cage) and the first full day of measurement (day 2) because birds were still getting used to the set-up and were not very active.

To compare the change in daily activity onsets (reflecting both the after-effects and the free-running period), we used mixed-effect models with the time of the onset of activity (in minutes after midnight) as the response variable and relative experimental day (trend over time), relative experimental day squared (for testing nonlinear changes) and site (city or forest) as explanatory variables. We also included the interactions between site and relative day squared and site and relative day to test whether the change over time in the onset of activity differed between sites. To account for the fact that we were using multiple measurements of onset of the same individual, we included the individual identity as a random effect. Finally, we also corrected for bird age (second calendar year or older), for the calendar day the bird arrived in the laboratory, for the actual light intensity value measured in the cage (min 0.3, max 0.6 lux) and also included a term containing the cage 'group' which accounts for the neighbour cages in the room. Once more, terms were tested using a Kenward–Roger approximation for *F*-tests.

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

(iv) Onset of activity and endogenous period

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

3. Results

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

(a) Activity patterns and repeatability of the activity parameters

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

Birds from different sites did not differ in average offset ($F = 0.07$, $p = 0.79$, city estimate = −124.58 ± 6.09 min, forest

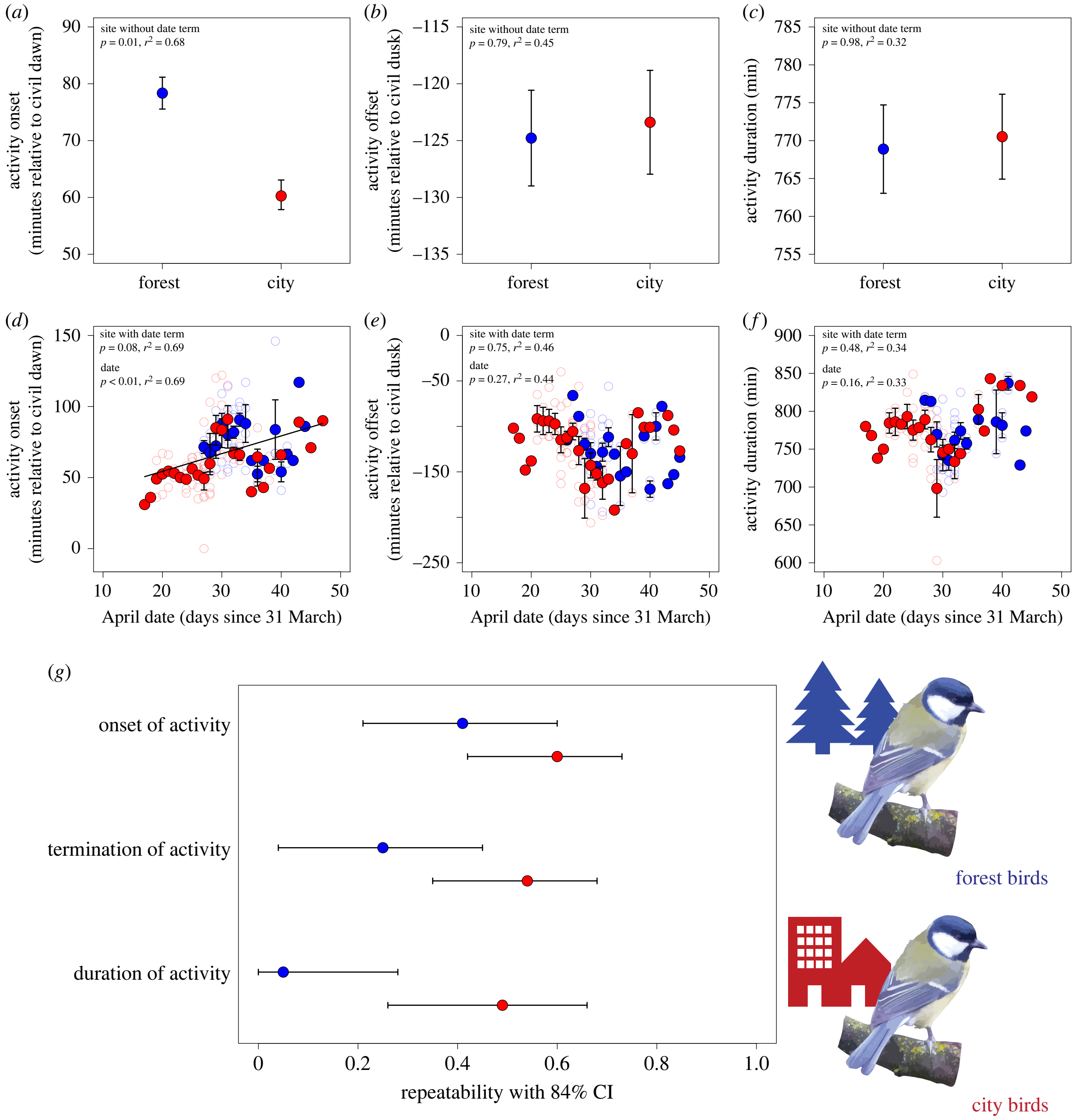

Figure 1. Activity patterns and repeatability of activity during incubation in city and forest birds. Raw mean values and standard errors per site for (*a*) activity onset (*b*) activity offset and (*c*) activity duration. Change over the season in (*d*) activity onset (*e*) activity offset and (*f*)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

estimate = −127.11 ± 6.98 min; figure 1*b*) or duration of activity ($F < 0.01$, $p = 0.98$, city estimate = 769.81 ± 6.81 min, forest estimate = 770.01 ± 7.73 min; figure 1*c*) regardless of the presence of the date term in the model. Moreover, there was no measurement date effect, with a similar offset ($F = 1.23$, $p = 0.27$, estimate = −0.81 ± 0.72 min; figure 1*e*) and duration ($F = 2.06$, $p = 0.16$, estimate = 1.22 ± 0.84 min; figure 1*f*) of activity throughout the season.

The onset of activity had high repeatability in both city ($R = 0.61$) and forest ($R = 0.43$) birds, while for offset and duration of activity repeatability was high ($R = 0.54$, $R = 0.49$) in city birds and moderate ($R = 0.21$) and low ($R = 0.05$) in forest birds, respectively (figure 1*g*). For all traits, there was an overlap between the 84% CI, thus the repeatabilities were not significantly different between sites (see [37,38,41]).

(b) Clock properties of city and forest birds

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

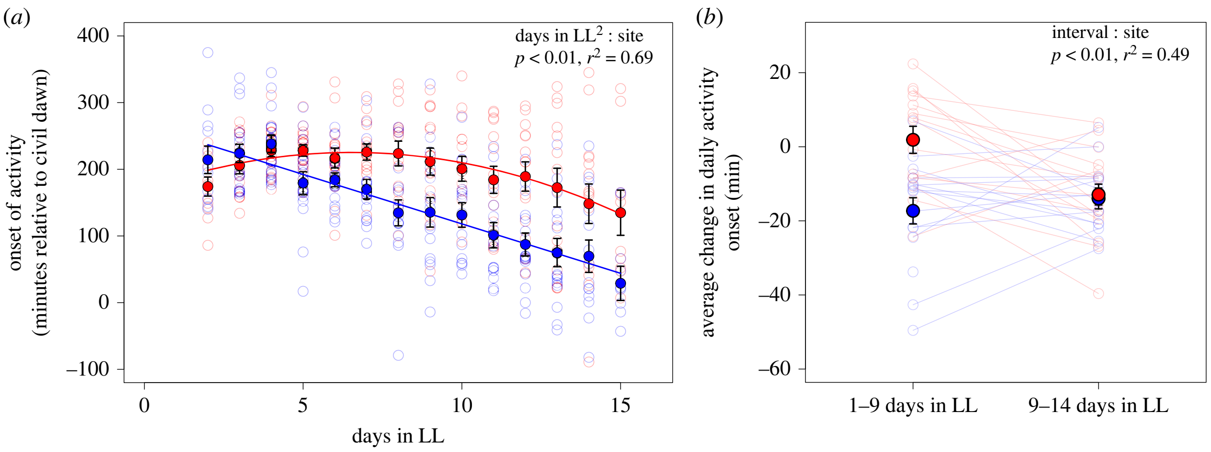

Figure 2. Clock properties of city and forest birds. (*a*) Change in activity onset (in minutes relative to civil dawn) of birds kept in constant conditions in the laboratory (LL). (*b*) Means and standard errors of the period length of urban and forest birds measured between 1 and 9 days and between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

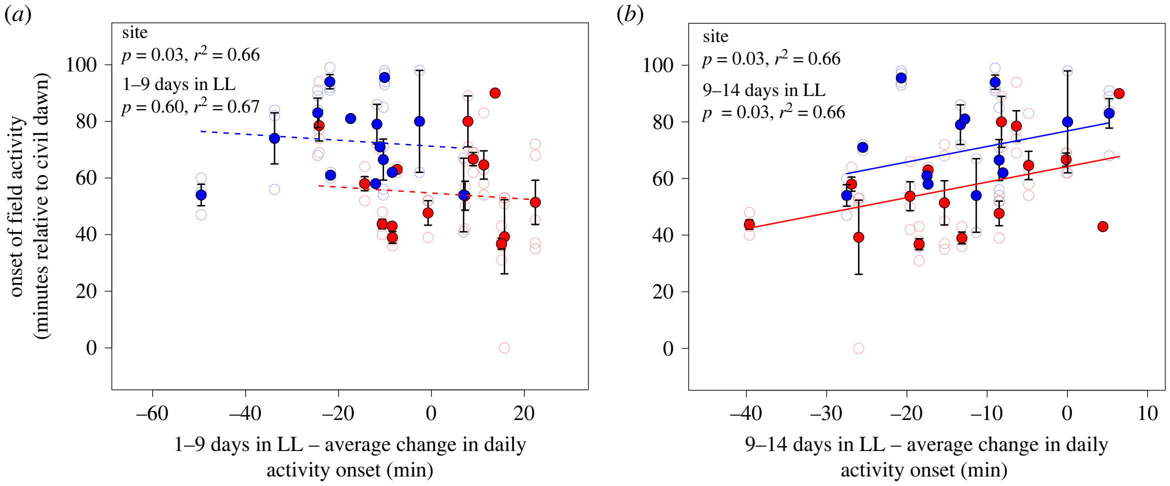

Figure 3. Relationship between onset of activity in the field and clock properties. (*a*) Activity onset relative to period length measured from 1 to 9 days in constant conditions in the laboratory (LL). (*b*) Activity onset relative to the period length measured from 9 to 14 days in constant conditions in the laboratory (LL). Activity onset is given as minutes relative to civil dawn, period length is given as change in minutes in daily activity onset in minutes relative to 24 h.

cycles after being placed in constant conditions (city: 106.71 ± 120.73 min from civil dawn; forest 210.10 ± 126.21 min from midnight), a pattern that was eventually reversed when forest birds further drifted from 24 h (figure 2*a*).

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

(c) Relationship between onset of activity and clock properties

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

4. Discussion

In this study, we showed that city and forest birds while having high repeatability in their onset of activity, do not differ in the timing of their activity patterns when date effects are taken into account. We also showed that city birds, despite having a similar circadian period compared with forest birds, had a stronger history dependency in their circadian period to the previous condition. This is expressed by a difference in retention of the approximately 24 h interval between consecutive onsets of activity after taking forest and city birds from the external light–dark cycle into constant (time-isolated) conditions in the laboratory. There, on average, forest birds directly revert to their internal, endogenous circadian period whereas city birds needed roughly nine circadian cycles to reach a circadian period of similar duration.

A difference in onset of activity between free-ranging forest and city birds, with city birds starting their activity earlier compared with forest birds, is in line with previous comparative studies between urban and non-urban birds. Such differences were observed in blackbirds (*Turdus merula*, [24]) an effect linked to artificial light at night. Indeed, blackbirds that are individually exposed to higher artificial light levels start their activity earlier compared with birds exposed to less light [12,18]. In the laboratory, low-intensity light at night strongly advances activity patterns in great tits (*Parus major*) in a dose-dependent manner [13,43]. Blue tits with (part of) their territory illuminated by light posts started their activity much earlier compared with birds in dark (forest) territories ([44], but see [45]). However, in our case, the difference we found in daily onset of activity may well be related to the earlier start of breeding in city birds, as the difference in daily onset disappeared with the inclusion of date in the model. Birds breeding earlier in spring may advance foraging activity just because of the relatively short natural day length; a comparable effect has been shown for the dark phase in experimental photoperiods for rodents in the laboratory [46]. Whether the artificial light at night in urban environments in fact facilitates earlier foraging by disturbing the perception of day length in great tits remains to be tested.

One of the challenges in the field of Chronobiology is how ubiquitous clock properties govern activity in the 'real world'; for example, how variation in tau is connected to behavioural or physiological differences [47]. Here we found a correlation between tau and activity, where shorter individual circadian period length is linked to an earlier onset of daily activity. This is in line with the expectations set by previous studies (e.g. [48,49]), although this effect was not found in an earlier study done in the same species [50]. Interestingly, in our study, the outdoor daily onset of both forest and city birds could be linked to circadian period as measured in the laboratory. In an earlier study, a comparable link was observed between circadian period and daily onset in city, but not in forest blackbirds [24]. Differences between this and our study could be due to site differences (e.g. variation in ALAN), species-specific clock properties, ecological differences (e.g. great tits are smaller and breed in cavities) or differences in response of males and females (their study only used males and we only used females). The males of our incubating females could have started their activity earlier in the cities when compared with forests as it is known that artificial light has a strong effect on dawn song onset in great tit males when defending their territory [51]. Seasonal timing of females may in turn be affected by males via their song activity, which has been suggested to have behavioural and physiological effects on female breeding phenology [52,53]. Finally, tau was measured at a different reproductive stage than activity (post-incubation) and seasonal effects could impact the absolute tau value. Such effects, however, should not have modified the relationship between tau and activity, where early birds have shorter and late birds longer tau values.

We can only speculate why city birds show after-effects, and forest birds do not. Similarly to differences in tau, it is unknown what the after-effects would mean for an organism in the wild, but the ubiquity of such clock property makes such speculation interesting. On one hand, the differences in after-effects could be a result of a (genetic) difference in the clock itself of city and forest birds. So-called after-effects have been extensively reported at the behavioural level [54]. Likewise, after-effects in internal clock speed have been found at the cellular level [55–57], but its underlying molecular mechanism is still not fully understood (but see [58]). Despite that, the general idea is that after-effects originate from a more stable phase relationship between multiple putative oscillators in the circadian pacemaker. These phase relationships may be established by prior entrainment and cause the clock to be more stable [27,28,59]. Moreover, a clock with a period closer to 24 hours is argued to be more stable and precise [60]. From a functional prospect, such a robust circadian clock, precisely resonant with the external cycle, could be beneficial in city environments, as the sharp contrast between the light and dark of the natural cycle is blurred by high levels of artificial light in cities at night. This is supported by the relatively high repeatability values of activity patterns that we found for the city birds. Thus, a circadian clock that is intrinsically more stable and less responsive to the aberrant and potentially confusing light pattern in cities is potentially adaptive.

While not a measure of light sensitivity *per se*, the literature on circadian rhythms provides support for the hypothesis that the difference in after-effects could indirectly represent differences in light sensitivity due to a more stable clock. For example, when birds are exposed to higher (constant) light intensities in the laboratory, the period of their circadian rhythm shortens (one of the 'Aschoff rules', [26,48]). Following this logic, if the forest birds are more sensitive to light, and even when exposed to only 0.5 lux in the laboratory, they would still experience it as brighter than city birds, resulting directly in a shorter tau. City birds, less sensitive to the constant dim light conditions, would only start shortening tau after a sensitization of the circadian system to light. Similarly, the lower sensitivity to light of city birds is also supported by behavioural studies where forest great tits responded more strongly to light at night by starting onset earlier in the laboratory [61], moreover, the sensitivity of the great tit circadian system to light is similar to what has been reported for other species. In an environment with less contrast between day and night and fluctuations in ALAN, a more stable clock, less sensitivity to perturbations of light could be beneficial. This contrasts with Dominoni *et al.*'s [24] findings, where the weaker city zeitgeber is suggested to select for alternative activity patterns (reduced clock robustness). Our data for city birds, where individuals maintain approximately 24 h rhythms for several cycles after being placed in constant conditions do not support less robust clocks in city birds.

Alternatively, the disparity in after-effects between city and forest birds could originate from non-clock differences, and result from a direct modulation of the circadian system in response to the light. For example, via a difference in perception of day length of city and forest birds. The effect of photoperiod in prior entrainment on after-effects has been experimentally shown [54]. Here, such differences between city and forest birds may be present and the result of the excessive additional artificial light in the dark phase of the urban cycle. A difference in perception of day length is plausible, as it may cause city birds to breed earlier in the season as we observed. This is in line with birds breeding earlier in response to controlled experimental light in forests [62]. It is also possible that there is a difference in sensitivity to light between city and forest birds as reported above, but this difference originates from effects on the input pathway to the clock, rather than on the clock itself.

A yet separate explanation could be related to stress coping mechanisms. Stressful conditions of cities could have selected mechanisms that allow birds to live in such environments [63] and in turn have effects on the rhythms measured in laboratory (in isolation). In earlier studies, a possible link between rhythmicity and response to social isolation (stress) has been shown in quail [64]. Since we measured birds solitary in cages for obtaining the clock properties measurements, we cannot fully rule out a potential effect of stress on the circadian organization, although great tits live in a different social structure compared with quail.

From a broader perspective, our study highlights the potential of comparing city and forest organisms to study how biological clocks may evolve in response to urban environments. An important, remaining question is whether the differences in the circadian properties between city and forest birds observed here are caused by local adaptation of clocks, or are merely a phenotypic response. There is evidence that genetic change can be rapid in cities [65–67], and the phenotypic differences between city and forest birds could indeed reflect genetic differences in the response of their clocks to light. However, the high plastic nature of activity patterns and the reversible nature of clock properties (e.g. after-effects can be easily induced by differences in the perceived photoperiod, [54,58]) suggest a stronger role of plasticity rather than genetic change. Such plasticity would indicate the ability of the circadian system to cope with rapid alterations in the light environment caused by urbanization. This would be important in cities as a circadian system that continues to run with a period of 24 h would retain the highest accuracy even when exposed to noisy urban cues [60], allowing animals to keep track of time. This in turn could lead to higher survival, as animals would better predict challenging moments of the day/night cycle [68,69]. The separation between phenotypic plasticity and genetic changes is, however, not trivial and requires specific experimental approaches in future studies.

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

Data accessibility. All data supporting this manuscript are available at: https://dataverse.nl/dataset.xhtml?persistentId=doi:10.34894/8KYFYO [70].

Supplementary information is provided in the electronic supplementary material [71].

Authors' contributions. B.M.T.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing—original draft, writing—review and editing; F.T.: investigation, methodology, writing—review and editing; K.S.: conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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