

Great Tit Activity Timing: Effects of Artificial Light at Night and the Spatial Habitat

Nadieh Reinders

S3449564

15-07-2021

Minor Thesis B-track Behavioural and Cognitive Neurosciences
Supervised by prof. dr. Barbara Helm and PhD student Aurelia Strauß
Biological Rhythms of Natural Organisms
Groningen Institute for Evolutionary Life Sciences (GELIFES)
University of Groningen



Abstract

Artificial light at night (ALAN) has rapidly increased all over the globe, and is associated with shifts in activity rhythms of many organisms. Since an accurate timing of activity is crucial for fitness, it is important to understand activity rhythms of species living in a rapidly changing world. A shortcoming of previous studies on bird activity is shortage of data outside the breeding season, and it remains unknown how the timing of activity relates to the spatial habitat. Therefore, the current study focused on the diel timing of Great tit activity during winter, and related this besides ALAN also to the distance to the nearest building. Nest boxes, in which birds roost, were distributed over a gradient of ALAN and urban characteristics. Newly developed activity loggers were used to measure bird activity and compared to radio telemetry techniques. The relationship of ALAN and the distance to the buildings with daily activity onset, offset and duration was analysed in a linear mixed model. Despite a limited sample size, we found indications of a 3.57 min earlier onset and a 4.38 min later offset of activity per unit increase in light intensity. The duration of the active phase tended to become 4.42 min longer. There were tendencies of a 7.27 min advanced onset, a 2.24 min delayed offset, and a 16.32 min longer duration per unit increase in distance from the nearest building. So, this pilot study showed that both ALAN and the spatial habitat may shift the diel timing of Great tit activity. Overall, the effects of ALAN fitted the expectations, but the effect on the offset was larger than suggested by previous research. The reason for the effects of the spatial habitat on the timing of bird activity remains not understood, but it opens interesting avenues for further research. Previous research indicated that shifted rhythms have negative health consequences, which may form an additional challenge where wild birds have to cope with. Studies like ours are thus needed to better understand the effects of anthropogenic environments on nature, in order to develop biodiversity friendlier urban areas.

See https://drive.google.com/file/d/1mODhJqzUS1Fs6c_1e4uhLQOinGNTK3Kn/view?usp=sharing for an animated summary video (but note that the content of this video has a slightly different emphasis).

Table of contents

Introduction	4
Methods	6
Study species	6
Study area	6
Nest boxes	6
Data collection	7
Light intensity	7
Loggers	7
Telemetry	9
Receivers	9
Tagging birds	9
Locating birds	10
Spatial data	10
Data processing and analyses	10
Loggers	10
Data processing	10
Calculating the onset, offset and duration of the active phase	10
Telemetry	12
Data processing	12
Calculating the onset, offset and duration of the active phase	12
Statistical analyses	13
Analysis logger data	13
Comparison of logger and telemetry data	13
Results	14
Logger data: effects of light intensity, distance to the nearest building and date	14
Comparison of logger and telemetry data	18
Discussion	20
References	23
Supplement	26
Acknowledgements	42

Introduction

Since the first light bulb illuminated the streets of New York city in 1879, the use of artificial light has rapidly spread over the world (Hughes 2004). Today, over 80% of the world and even 99% of Europe and the United States is illuminated by artificial light at night (ALAN), as visualized in *Figure 1* (Falchi et al. 2016). ALAN can come from direct light sources, such as streetlights, vehicles and billboards. Furthermore, it can be the result of reflections from sealed surfaces and buildings, and atmospheric scattering of light. ALAN illuminates places that were previously dark, at timepoints where the natural sunlight has never shined, and with spectra and intensities different than those of the sun and moon (Gaston et al. 2014a, 2015). For instance, the illuminance of a full moon on a cloudless night is in the order of 0.1 lux (Rich and Longcore 2006), but direct lighting under street lights can easily become 40 lux, and stay 1 lux over 20 m away (Bennie et al. 2016). Besides the changes associated with human settlement, industry, and transport networks, this rapid increase of light has profoundly altered the natural light-dark cycle. Many anthropogenic changes of the environment (e.g. changes in temperature and CO²) have historical analogs, meaning that we can learn from previously naturally occurred changes. However, the effects of ALAN are unprecedented (Gaston et al. 2014a).

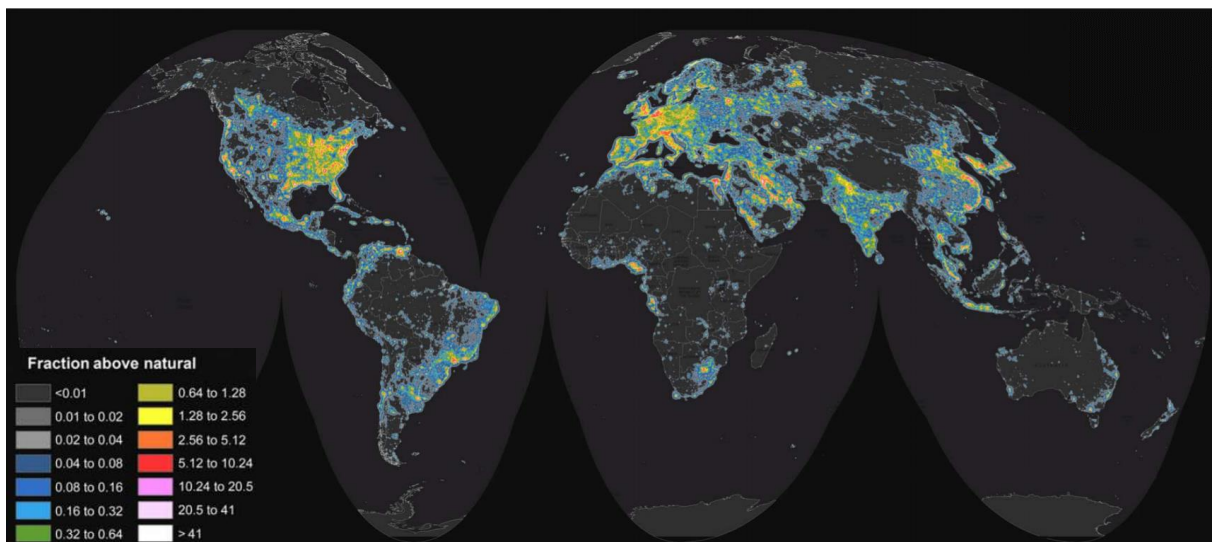


Figure 1: Artificial sky brightness across the globe. Many places of the world, and especially Europe and the United states, are illuminated by artificial light at night. Artificial sky brightness is shown as a ratio to the natural sky brightness (which is around 174 mcd/m²) in twofold increasing steps. Adapted from Falchi et al. (2016).

Artificial lighting brought many social and economic benefits for humankind (Gaston et al. 2014b, Ebbensgaard 2015), and also nature can benefit from it in some ways. For instance, animals can occupy illuminated spots to have extended foraging opportunities (Lebbin et al. 2007, Dwyer et al. 2013, Pugh et al. 2016). However, ALAN has also its costs. On our rhythmic planet, organisms have evolved systems that are highly sensitive to light. Light is a key player for vision, organismal development, growth and survival. It is important for phototaxis, phototropism, and synchronization of circadian and circannual clocks (Falcón et al. 2020). Our rapidly altered light environment is therefore a threat for the refined functioning of light sensitive systems and their major implications (e.g. across food chains and communities).

The negative effects of ALAN are widespread. They cover a broad range from changes in genes, to organismal physiology and behaviour, to ecosystems (Sanders et al. 2020). For instance, a recent study reported that the endocrine, reproductive, and neural development of *Aquatica ficta* firefly larvae could be impaired, due to differentially expressed genes in these systems after ALAN exposure (Chen et al. 2021). Another study recently reported that birds avoided noisy backyards, which was even more pronounced when excessive light was also present (Wilson et al. 2021). Two important negative effects can be categorized as 1) attraction to artificial light sources and 2) changes in circadian and circannual rhythms. Well known examples of the first category are migratory birds which are trapped

by the light beams of the 9/11 memorial (Van Doren et al. 2017), and misorientations of sea turtle hatchlings by on-shore lights (Truscott et al. 2017). The second category is based on the circadian and circannual system, that anticipates environmental fluctuations, and enables organisms to align the timing of their physiology and behaviour with the natural light-dark cycle (Foster and Kreitzman 2005, Helm et al. 2017). In this way, the disruption of the natural light-dark cycle by ALAN can change many rhythms in almost all organisms, with possible consequences for health and fitness (Vetter 2020, Moreno et al. 2020).

Shifts in daily activity rhythms are common effects of ALAN. These are well studied in diurnal birds, because birds are abundant and detectable. Furthermore, it is easier for us, humans, to study animals that are active around the same time as we are. Shifts in activity patterns are shown in both free-living (Dominoni et al. 2014, Ouyang et al. 2017) and captive birds (Spoelstra et al. 2018, Dominoni et al. 2020). The most prominent shift is the advance of morning activity onset (Kempnaers et al. 2010, Dominoni et al. 2014, 2020, Raap et al. 2015, de Jong et al. 2016, 2017, Spoelstra et al. 2018, Bermúdez-Cuamatzin et al. 2020). A shift in evening activity offset is less established. Some researchers reported a delayed offset with increasing light intensity (de Jong et al. 2016), some a less pronounced effect (Da Silva et al. 2014, de Jong et al. 2017, Dominoni et al. 2020), and others no effect (Dominoni et al. 2014, Raap et al. 2015). Most observational studies on free-living birds were done during the breeding season. Less is known about the activity patterns of wild birds outside the breeding season (but see Raap et al. (2015)). Thereby, the timing of activity may not only be affected by ALAN, but also by other factors, such as spatial characteristics of the habitat in which an animal lives. Gaston et al. (2015) identified a lack of studies that related the timing of activity to the spatial habitat, and proposed this topic as a research challenge for future studies. To date, not much research has been devoted to this.

This pilot study aimed to fill these gaps by 1) investigating the effects of ALAN on the diel timing of bird activity during winter, and 2) exploring how the spatial habitat - the distance from the roosting site to the nearest road and building - covaried with this timing. I focused on the Great tit (*Parus major*), which is a rewarding study species. Great tits are like humans diurnal (de Jong et al. 2016), and use nest boxes for roosting (Krištín et al. 2001). They are already well-studied with respect to ALAN (see "*Study species*"), and their abundance in and acceptance of human modified habitats (Hoyo et al. 1992) allows to study the effects of ALAN on free-living animals. The Zernike Campus of the University of Groningen in the Netherlands was used as a model study system. Great tits are abundant birds at and around the campus (see <https://www.vogelbescherming.nl/tuinvogeltelling/resultaten>), and the urban characteristics and varying intensity of ALAN at the campus made this a good location to study the effects of ALAN and the spatial environment in an urban habitat (for details see "*Study area*").

We hung wooden nest boxes on trees at the campus, of which the locations covered a large variation of ALAN intensity and environmental factors. We developed a novel technique, based on an infrared movement detector, to measure bird activity in these nest boxes. Additionally, we used radio telemetry to investigate how activity data from this conventional technique for measuring animal activity behaviour (Lövy et al. 2013, Dominoni et al. 2014, Nord et al. 2016, Schofield et al. 2018, Jarrett et al. 2020) matched the activity data of our novel approach.

In line with previous research, it was hypothesized that birds roosting in a nest box with a higher light intensity have an earlier onset of daily activity. I expected a later or unchanged offset of daily activity with increasing ALAN. The duration of the active phase, which was based on the difference between the offset and onset time, would consequently be longer with increasing ALAN.

Methods

Study species

The Great tit is a common passerine bird in the family Paridae, with a wide distribution across much of Eurasia, including populations in North West Africa and the Middle East. The bird is 12.5 to 14.0 cm in length, and is characterized by its yellow breast with a vertical black stripe, a black cap and white cheeks. The Great tit is originally a forest bird, but it has adjusted to the anthropogenic landscape and can be very common in urban areas (Hoyo et al. 1992). Furthermore, it is diurnal (de Jong et al. 2016) and accepts nest boxes easily (Kvist et al. 2003). Besides breeding, Great tits use nest boxes for roosting too. Krištín et al. (2001) studied nest box occupancy during October-April and found a maximum nocturnal occupancy of 64% in November, which slightly decreased up to April.

Due to their broad range, diurnality, abundance and use of nest boxes, Great tits have been a model species for research in evolutionary, ecological and behavioural research for over 50 years (Laine et al. 2016). Most studies have focused on the populations in north-west Europe, and so did this study. The Great tit is also well studied with respect to the functioning of its circadian and circannual clock, and its behavioural responses to ALAN. For instance, Sun et al. (2017) experimentally exposed wild Great tits to ALAN inside their nest boxes. They found that the wake-up time and the time of leaving the nest boxes was earlier, that the birds fell asleep later and slept less. Great tits are most sensitive to white light (Ouyang et al. 2017, Ulgezen et al. 2019), and a shift in activity patterns is already caused with low light intensities (~1.5 lux) (Raap et al. 2015, de Jong et al. 2016, Spoelstra et al. 2018, Ulgezen et al. 2019, Dominoni et al. 2020). Placing this into perspective: white light is the colour which is emitted by amongst others street lights and billboards, and the light intensity 20 m away from a street light is still 2 lux (Bennie et al. 2016).

Study area

The current study was conducted at the Zernike Campus of the University of Groningen in the northern Netherlands (53°24'N, 6°53'E), during the winter of 2020/2021. The Zernike Campus is located at the edge of the city, and has a separation between research based companies (north) and academic buildings (south). The northern and southern parts are both approximately 100 hectare. At the campus are small fields, hedges, trees and watery areas (see *Figure 2*). These provide a home for some insect and animal species, amongst others the Great tit. The east and north sides of the campus are surrounded by small pieces of forest and agricultural fields. South is the city of Groningen and west of the campus is the canal and urban district "Reitdiep". The campus is well accessible by car, bus and bike. The main roads, parking spots and sealed surfaces are illuminated by street lights. Together with light from the buildings and sports fields, these streetlights cause the campus to be illuminated by intermediate amounts of ALAN. The average sky brightness (SQM) at the Zernike Campus is 19.91 mag./arc sec², and can locally be around 17.00 mag./arc sec² on a scale from 22 (the darkest sky) to 16 (the brightest sky). For comparison: the average sky brightness in our capital Amsterdam is 18.19 mag./arc sec², and on the naturally dark Wadden island Schiermonnikoog 21.76 mag./arc sec² (see Sánchez de Miguel et al. 2017, van Hasselt et al. 2021, lightpollutionmap.info and washetdonker.nl). The spatially varying intensity of ALAN and the urban features make the Zernike Campus a good location to study the effects of ALAN and the spatial environment in an urban habitat. Note that the Zernike Campus is usually a crowded place, but due to the Covid-19 lockdown, human activity was really low during the course of this study.

Nest boxes

In November 2020, we distributed 92 wooden nest boxes across the southern part of the campus (*Figure 2*). Nest box locations included a large variation of ALAN, cardinal directions of the entrance hole, and environmental factors (such as vegetation and distances to roads and buildings). The boxes were hung on trees with an average height of 2.30 m. The dimensions of the inside of a box were 9.0*12.5*23.4 cm and the distance from the bottom to the entrance hole was 20.0 cm (*Figure S1*). The

entrance hole (2.80 cm in width) was idealized for the Great tit. However, this could not prevent that other small birds, such as the Blue tit (*Cyanistes caeruleus*), used these boxes. The coordinates of the nest boxes were measured as WayPoints in WGS 84 datum, using a Garmin eTrex 30x (Garmin Ltd., Olathe, US). For one of our nest boxes (number 133) the coordinates were not accurate. Therefore, the correct coordinates were established by dropping a pin in Google Maps at the location of the tree (<https://maps.app.goo.gl/VbosWTNZ1TnqgLZD9>).

Data collection

Light intensity

To approximate the amount of ALAN that was perceived by a bird, we measured the light intensity in front of the entrance holes with a lux meter (Unitest digital lux meter, Beha-Amprobe, Eindhoven, the Netherlands). These measurements were done from 14-17 December 2020, at and just after new moon, so when the moonlight had least impact on the measurements. Light measurements could not be done on the same day, due to time constraints. Measurements were done during astronomical darkness, so after astronomical dusk and before astronomical dawn when the sun was $>18^\circ$ below the horizon (*Figure S2*). Light was measured three times in a row for each nest box. The average of these values was reported as the light intensity.

Loggers

To measure nest box occupancy and the timing of bird activity, we developed loggers that detect movement in a nest box. A logger consisted of a wide-angled infrared movement detector (PIR sensor ST-00081, SimplyTronics, Washington, US), a power source (NP1.2-6 accu, Yuasa, Krefeld, Germany; 1.2Ah; 6V), and a data logger (EL-USB-5, Tempro, Brecht, Belgium). We constructed an aluminium frame that fits inside a nest box, and attached the elements to it by gluing and soldering (see *Figure S3* and *Figure S4*). The loggers functioned as a tracking system of bird activity in a nest box over time. When a bird was moving, the logger detected this and saved it as data with a timestamp.

Loggers were placed in the nest boxes in six sets (*Table 1*) between 16 December 2020 and 16 March 2021. The aim of all sets was to obtain activity data from the birds. An additional aim of set 6 was to have boxes with both a logger and a bird which carried a transmitter (see below), so logger and telemetry data could be compared. The nest boxes of set 1-4 were selected in a way that the number of loggers per set was equally distributed between nest boxes with a low (0-0.99 lux), medium (1-2.99 lux) and high (>3 lux) light intensity. The boxes of set 5 were the replacement nest boxes of the telemetry birds (see below). The nest boxes of set 6 were selected based on three telemetry detections (see below) in the boxes the night before. A logger was placed in each of the boxes of the three detections. There was an extra logger placed in the box next to each detection box, in case a bird would move to a different box, because of possible fear for the logger. This resulted in a total of six loggers for set 6.

Table 1: Overview loggers in nest boxes. Data loggers were put in nest boxes in six sets over time. Each set consisted of a different number of loggers, and was present for a different number of nights. The asterisks behind 5 January and the number of nights indicate that there were three loggers not present on 5 January (these were put in the nest boxes on 6 January), and therefore measured one night less.

Set	Date loggers in	Date loggers out	# Nights*	# Loggers present
1	16 Dec 2020	3 Jan 2021	18	30
2	5 Jan 2021*	16 Jan 2021	11	25
3	16 Jan 2021	19 Jan 2021	3	6
4	20 Jan 2021	1 Feb 2021	12	23
5	2 Feb 2021	23 Feb 2021	21	12
6	23 Feb 2021	16 March 2021	28	6

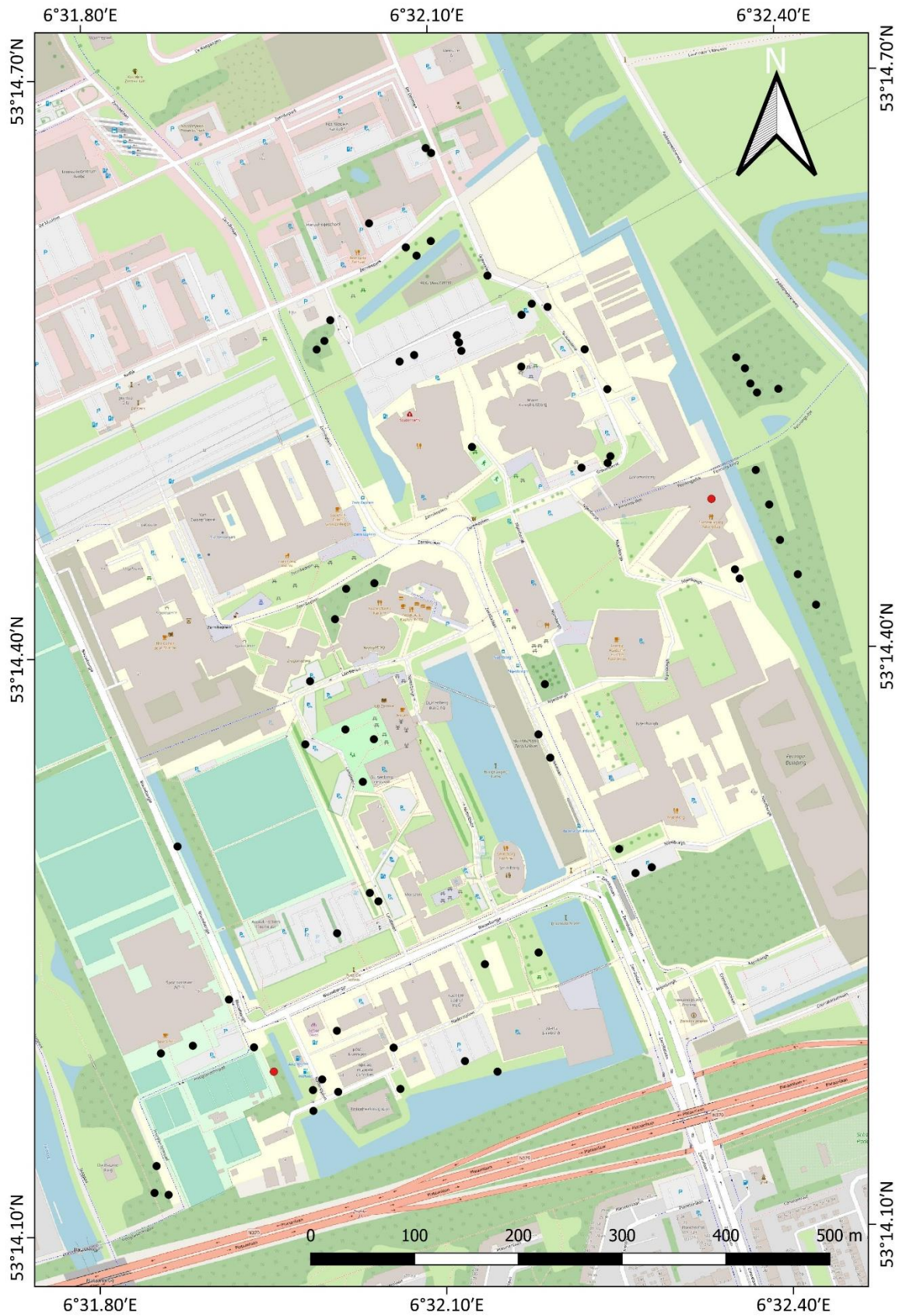


Figure 2: Location of all distributed nest boxes at the Zernike Campus. Black dots indicate the locations of the nest boxes and red dots indicate the locations of the stationary receivers (lower left Sports centre and upper right Linnaeusborg). The map was made in QGIS with an OpenStreetMap base layer.

Telemetry

Radio telemetry is a tool to locate animals, and to track their movement behaviour. Telemetry makes use of radio signals (electromagnetic waves). These signals are emitted by a small radio transmitter, which is attached to an animal. Each transmitter, and thus bird, has its own frequency, which allows to distinguish individuals. An antenna picks up the radio signals and a receiver processes the signal. Antennas and receivers can be both mobile and fixed. A mobile receiver records signal strength and transforms the radio signals into beeping sounds. The beeps become louder when the receiver gets closer to the animal wearing the transmitter. In these ways, with mobile receivers, animals can be located. The fixed receiver is used to collect data of the signals over time. Received signals have a constant, small, amplitude when the animal is not moving. In contrast, the signal fluctuates when the animal is moving. This characteristic can provide information about the temporal component of animal movement, i.e. activity patterns (see Dominoni et al. (2014) and Jonasson (2017)).

Receivers

A fixed receiver, SensorGnome, with five directional antennas pointing into five different directions (6-elements Yagi antenna, SIRIO WY 140-6N, SIRIO Antenne, Volta Mantovana, Italy) was present on the roof of one of the buildings at the campus (Linnaeusborg, 53°24'N, 6°54'E). A SensorGnome consisted of a PI3 (Raspberry Pi 3 Model B, Raspberry Pi Foundation, Cambridge, UK), SensorGnome software (SensorGnome Software Release 20.10.2018, SensorGnome Project, OpenSource), a dongle per antenna (FUNcube Dongle Pro+ (A20), FUNcube Dongle, London, UK), and a GPS (GLO-BU353S4, Globalsat Group, Boca Raton, US). A second SensorGnome with one omnidirectional antenna (SIRIO CX 148 U - 148-152 MHz "Fietspomp", SIRIO Antenne, Volta Mantovana, Italy) was attached to a tree close to the sports centre (53°24'N, 6°53'E) (Figure 2), based on where we found the birds with the mobile receivers (see "Locating birds") the day before. These fixed receivers provided data for the activity patterns of the birds. Both SensorGnomes scanned each frequency for 10 sec before switching to the next frequency, the max pulse length recorded was 25 msec, and the minimal signal to noise ratio was set to 3dbi.

Tagging birds

13 Great tits (15.8-18.8 g, 8 males, 5 females) were equipped with a temperature-sensitive radio transmitter (VHF Avian Tag: Pip (Ag317), Lotek, Wareham, UK; 0.47 g; 150-151 MHz) onto the upper back (Figure 3). The birds were caught while roosting inside the nest boxes during a complete roost check on 19 January 2021. For a parallel running experiment, the birds were brought for two weeks to the Netherlands Institute for Ecology (NIOO-KNAW) to measure the free running period in continuous dim light (ca. 0.5 lux). The birds were individually housed with food ad-libitum (for a similar method, see Spoelstra et al. (2018)).

The birds were tagged at the NIOO-KNAW on 4 February 2021, with a permit to Barbara Tomotani (NIOO-KNAW). A transmitter was sewn onto a small piece of cloth to improve adhesion. Some feathers onto the bird's upper back were trimmed to allow closer attachment of the transmitter to the skin. We used eyelash glue for attachment of the transmitter and a bit of super glue for extra adhesion (see Nord et al. (2016) for a similar procedure). After tagging, the birds were brought back at night to their capture nest box in Groningen. One bird was released in a different empty nest box, because this location had a higher probability of telemetry detections than the capture nest box. Two days after the release of the birds, one of the transmitters was found in a forest patch at the east side of the campus, so the final telemetry sample size consisted of 12 birds.

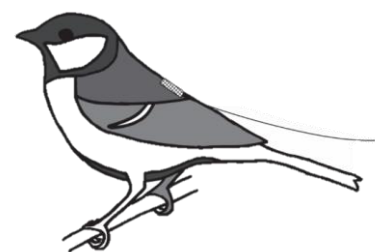


Figure 3: Schematic representation of a radio transmitter attached to the upper back of a Great tit. Adapted from Nord et al. (2016).

Locating birds

Roosting sites of the birds were located on four nights (6, 17, 22, 23 February 2021) using a mobile device consisting of a 3-elements VHF Yagi antenna (LITEFLEX, Lotek, Wareham, UK; 150MHz) attached to a VHF receiver (SRX800 MD2, Lotek, Wareham, UK). Transmitter frequencies were manually entered into the receiver. The birds were located by walking towards the strongest signal. The signal was followed until a high signal strength was obtained with a low gain, whilst pointing into the direction of a nest box. This nest box was then noted as the inferred roosting site of the bird.

Spatial data

A map was made in QGIS (version 3.16.3-Hannover, QGIS.org, 2021. QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>) to visualize the study area (*Figure 2* and *Figure 7*). Furthermore, spatial data were used to investigate the habitat features underlying the activity timing of the Great tits. Spatial data in the form of OpenStreetMap shapefiles were accessed via download.geofabrik.de.

The distances from the nest boxes to the nearest buildings and roads were calculated in QGIS. The reference coordinate system (CRS) and datum were set to Amersfoort / RD New, 28992, which is the recommended format for the Netherlands. Building and road shapefile layers were added to the project as vector data layers, and consisted of polygons and lines, respectively. A csv file with the coordinates of the nest boxes was also added, and in a subsequent step saved as a shapefile. The CRS and datum of the layers did not correspond to those of the project. Therefore, the layers were transformed to the CRS and datum of the project (CRS: Amersfoort / RD New, ESPG: 28992). The building and road data layers contained all data from the northern Netherlands, but there were only data needed for the Zernike Campus. Therefore, a polygon was drawn in the size of the campus, and the building and road data within the Zernike Campus polygon were selected by the *Select by Location* tool. The Feringa Building was already part of the building layer, however, the construction of this building was not finished yet. Therefore, this polygon was removed from the dataset. To calculate the distances from the nest boxes to the nearest buildings and roads, building polygons were first converted to lines with the *Polygons to Lines* tool. Lines of the buildings and roads were converted to points with the *Lines to points* tool. The distance between the points was set to 1 m. For each nest box, the distance to the nearest building and road was calculated with the *Distance to Nearest Hub (Points)* tool. See *Figure S5* for a flowchart of this process. Because there was no clear indication of the buildings and roads that could be most disturbing to the birds, any road or building was included.

Data processing and analyses

Loggers

Data processing

19 out of our 92 boxes were excluded due to logistic problems. These problems encompassed not working loggers or loggers that were turned off, loggers that displayed the wrong time, and nest boxes that fell off or disappeared. This resulted in a final sample size of 73 nest boxes.

Logger data were obtained with EasyLog USB software (version 7.6.0.0, Lascar Electronics Ltd., Salisbury Wiltshire, UK). The data were stored as 1 and 0 values over time. When there was a detection, the logger gave a state 1 value and a timepoint, after which it reset itself to a state 0 value after a few milliseconds to about a second (no fixed interval). Data was filtered on the state 1 values. This resulted in many datapoints when a bird was present and moving, and no datapoints when there was no presence or no movement. The plots that were made by the EasyLog USB software (see *Figure 4* for an example) allowed for the visual selection of suitable, occupied nights. There were in total 136 suitable nights.

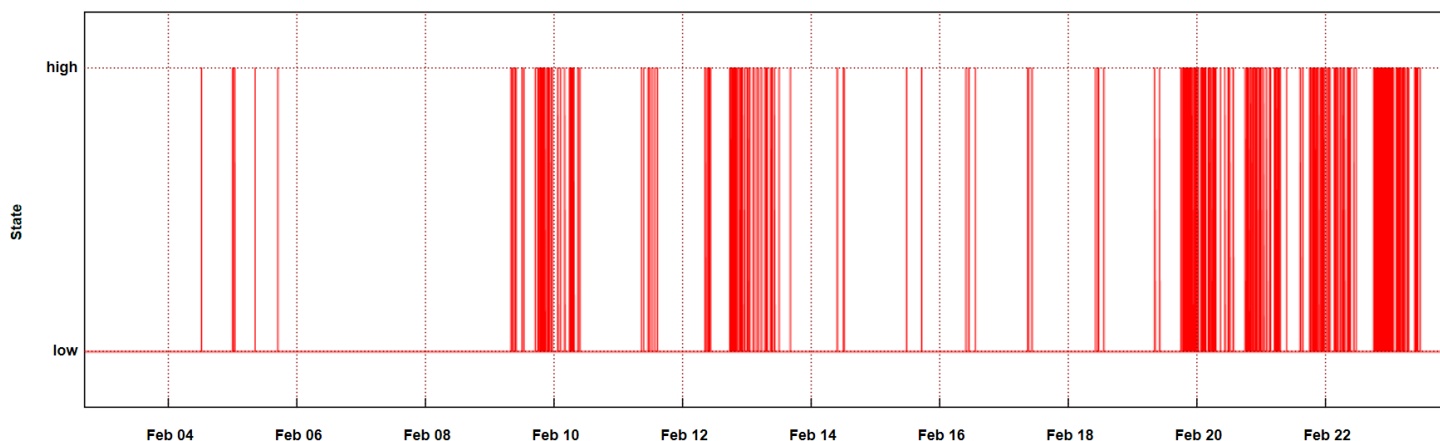


Figure 4: Example of a figure made by the EasyLog USB software. A red line indicates a detection of a bird. Many lines in a row indicate that the bird was present for a longer time. Looking at the patterns of many lines and no lines allowed for the selection of suitable nights for the analyses. In this example, there was a bird present on the night from 9 to 10 February, from 13 to 14 February, and the nights from 19 to 23 February. The data are from set 5, box 115, 3-24 February 2021.

Calculating the onset, offset and duration of the active phase

A self-made script in R (version 1.2.5033, R Core Team, 2019, Boston, MA, USA) calculated for each day per nest box the inferred onset and offset of activity. I defined the onset of activity as the latest activity timepoint in a time window after dawn, so just before the bird left the nest box. Similarly, the offset of activity was calculated as the first timepoint in a window before dusk, so just after the bird entered the nest box (Figure 5). The window was set to 0.65 h after dawn, and 1.10 h before dusk. See *Supplement Box 1* for more information about the selection of the best windows. The times of dawn and dusk were calculated in R, using the *suncalc* package (version 0.5.0, B. Thieurmel and A. Elmarhraoui, 2019). Here, the definition of dawn was when morning nautical twilight ended, and morning civil twilight started. Dusk indicated the end of evening civil twilight, so the start of the nautical twilight. In both cases, the sun was 12° below the horizon (Figure S2).

Onset and offset times were expressed in decimal hours relative to dawn and dusk, respectively. The times of dawn and dusk were subtracted from the times of activity onset and offset (e.g. *relative* $t_{onset} = t_{onset} - t_{dawn}$). In this way, a negative relative onset indicated a timepoint before dawn, and a positive relative onset a timepoint after dawn (similar for the offset). The duration of the active phase was calculated as the percentage of time the birds were active during the daylight fraction of the day ($duration_{active} = (t_{offset} - t_{onset}) / (t_{dusk} - t_{dawn}) * 100$).

Multiple detections in the same nest boxes were assumed to be from the same bird when there was an occupied night max 3 days later. It was assumed and treated as a different bird when there were more than 3 days in between the occupied nights. This rule divided logger data from 11 nest boxes in 15 birds. See *Table S1* for an overview of the division. Two onsets (bird 1, 1 January; bird 6, 14 January) and one offset (bird 3, 12 January) were removed from the data, because visual inspection of the output plots (see *Supplement Box 1* for an example) indicated that the calculated values were not the true values. The offset of bird 10 on 29 January was slightly later (11 min) than the output plot suggested. However, this one was not excluded because there were only two datapoints of this bird. See *Table S2* for an overview of the number of onsets, offsets and durations per bird.

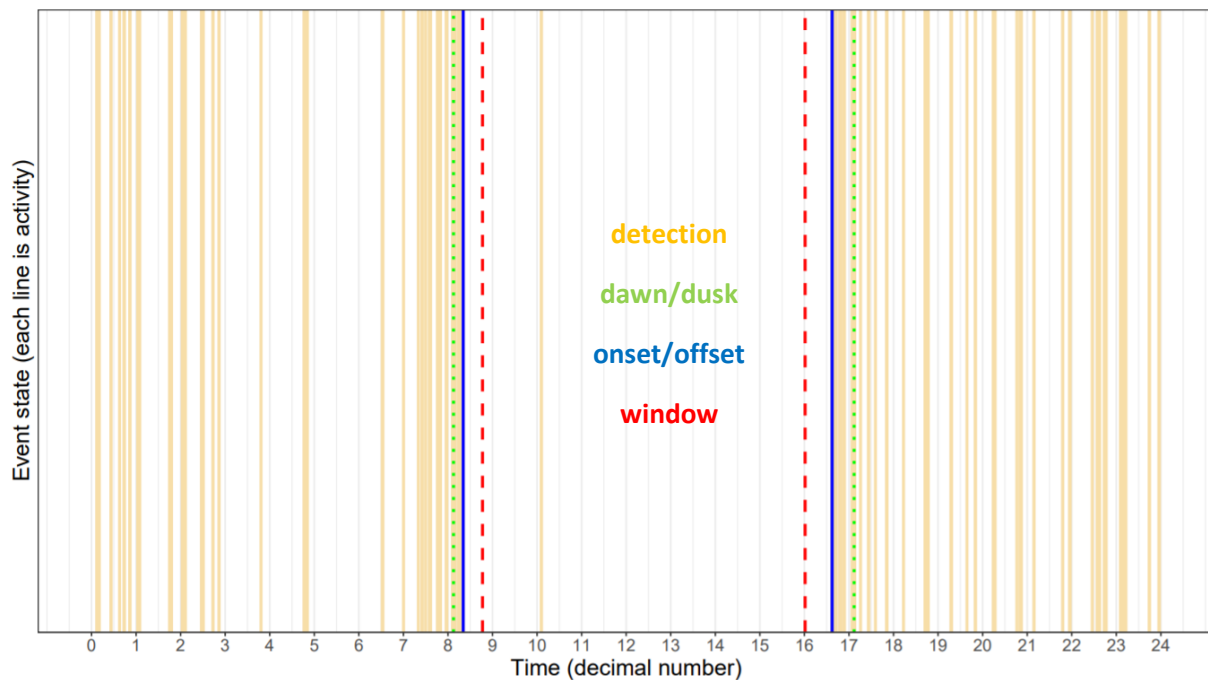


Figure 5: Example of the onset and offset of daily activity. The start and end of daily activity are typically characterized by many detections (apricot lines) in a short time, not many detections after this burst of activity in the morning, and not many detections before the burst in the evening. The onset (left blue line) was calculated as the latest datapoint in a morning window of 0.65 h (left red dashed line) after dawn (left green dotted line), so just before the bird left the nest box. The offset (right blue line) was calculated as the first datapoint in a window of 1.10 h (right red dashed line) before dusk (right green dotted line), so just after the bird entered the nest box. Dawn indicates the start of morning civil twilight and dusk indicates the end of evening civil twilight, so when the sun is 12° below the horizon. The data is from bird 1 (set 1, box 115, 28 December 2020).

Telemetry

Data processing

Telemetry data usually contains a lot of noise, due to interference of the signals and the detection of background noise. Therefore, several filtering steps were needed. First, the datapoints that fell within the second of frequency change were removed. Because the SensorGnome recorded multiple signals per second, the datapoints of low signal quality were removed by selecting only the detection with the strongest signal (lowest signal to noise ratio) per second. Subsequently, data was filtered by selecting the detections where the deviation of the frequency was between 1.5 and 5.5 Hz. Of the remaining data, differences in signal strength between consecutive time stamps per bird and per antenna were calculated, whereafter time was binned in 3-min intervals throughout the day. Each interval was either given a mean signal difference across multiple antennas or a NA value (when no data was available for that interval).

Calculating the onset, offset and duration of the active phase

A behavioural change point analysis (BCPA) was used to calculate the onset and offset of activity with the telemetry data. This method was developed by Gurarie et al. (2009) and successfully used by Dominoni et al. (2014). The model, which was written in R, assumed that the change in signal strength across time was drawn from two different normal distributions (one for the active and one for the non-active state) within a temporal window of 4 h before and 2 h after dawn/dusk. An Akaike information criterion (AIC) value was given to all possible change points in activity status. The timepoint that produced the lowest AIC value was identified as the onset/offset of activity. The onset/offset of daily activity was not calculated when the temporal window contained less than 84 datapoints (70%).

Filtering steps of the data and window selections for the BCPA were not optimized, like it was for the logger data. As a result, the telemetry onsets on 22 and 25 February were and the offset on 22

February was different than suggested by the output plots. These were therefore corrected by visual inspection of the data (see *Supplement Box 2*). Furthermore, we encountered an intractable apparent mistake in the timestamp or time zone, which affected all onsets and offsets from 24 February onwards (about 65% of the data). As shown in *Table S3*, it looked like the telemetry onsets and offsets shifted 1 h backwards compared to the days before, which was not seen in the logger data. To correct for this, telemetry onset and offset times from 24 February onwards were shifted 1 h forward. These corrected data were used for subsequent analyses, unless otherwise indicated.

Statistical analyses

Analyses logger data

The onset, offset and duration of the active phase of the logger data were analysed in additive linear mixed-effects models, using the *lme4* package (Fitting Linear Mixed-Effects Models Using {lme4}, Bates et al., 2015) in R. Light intensity, distance to the buildings, and date were treated as fixed effects. The light intensity and distance to the nearest building were transformed by the natural logarithm, because both distributions were skewed to the left. During our study period, we experienced an extraordinary drop in ambient temperature, followed up by a peak (see *Figure S6*). Therefore, besides bird number, the average daily temperature was also treated as a random effect in the models. Temperature data from the closest weather station in Eelde (about 15 km south of the Zernike Campus) was obtained from the Royal Dutch Meteorological Institute (KNMI, via <https://www.knmi.nl/nederland-nu/klimatologie/daggegevens>). Repeatabilities (intraclass correlation coefficients) of the random effects were calculated by dividing the random effect variance by the sum of the random effect variance and the residual variance ($r = \sigma_i^2 / (\sigma_i^2 + \sigma_\epsilon^2)$). The interpretation of repeatability values followed the guideline of Koo and Li (2016). All models were checked for a normal and homogenous distribution of the residuals, via quantile-quantile plots and Shapiro-Wilk tests. As suggested by Wasserstein and Lazar (2016), more attention was paid to the effects than to the p-values.

Correlations between the variables were checked for model optimization. The log of the distance to the nearest building was only weakly correlated with the log of the light intensity (linear regression, $\beta = -0.56$, $F_{1,9} = 1.56$, $p = 0.24$, $R^2 = 0.15$, Pearson's $r = -0.39$), and so was the log of the distance to the nearest road (linear regression, $\beta = -0.54$, $F_{1,9} = 3.85$, $p = 0.081$, $R^2 = 0.30$, Pearson's $r = -0.55$). However, there was a strong correlation between the log of the distance to the nearest road and the log of the distance to the nearest building (linear regression, $\beta = 1.09$, $F_{1,9} = 10.43$, $p = 0.010$, $R^2 = 0.54$, Pearson's $r = 0.73$), as shown in *Figure 6*. Therefore, either the distance to the buildings or the distance to the roads had to be removed from the models. Onset, offset and duration models were run, and each time one of the distances was excluded. The two models per onset, offset and duration were compared by giving them an AIC value via the *AIC()* function in R. An average AIC value was calculated in order to decide which distance type should be excluded from the models. Since the average AIC value without the distance to the road was lower, so better, this variable was excluded from all subsequent analyses (*Table 2*). So, for example, the final model for the onset became:

```
OnsetModel <- lmer(Onset~LogLight+LogBuilding+NumericDate+(1|Bird)+(1|AvgT),data=OnsetData).
```

Since the slope estimates of the light intensity and distance to the nearest building were on a natural logarithmic scale, it was not possible to get a generalized slope estimate for the non-log variables. Namely, the log was used to turn skewed distributions into less skewed ones. The models I used looked for a linear effect based on these less skewed distributions. If one converts the slope to a non-log scale, the linear effect on the log scale disappears, i.e. the slope becomes different for each value of x . A solution is to look at the slope estimate in terms of changes in x , so basically the other way around. Given that the interpretation of the slope is the increase in y with a one-unit increase in x , we can apply some mathematics and say that $\log x + 1 = \log x + \log e = \log(ex)$. In other words, when $\log x$ is increased by 1, the same slope is obtained by multiplying x by e (which is approximately 2.72). Since $(2.72 - 1) * 100 = 1.72$, one could also say that the slope on the log scale is the same slope on the non-log scale when x increases by 172%. So, the interval to the next non-log x value is shorter for small values of x , and longer for large values of x , but the slope remains similar.

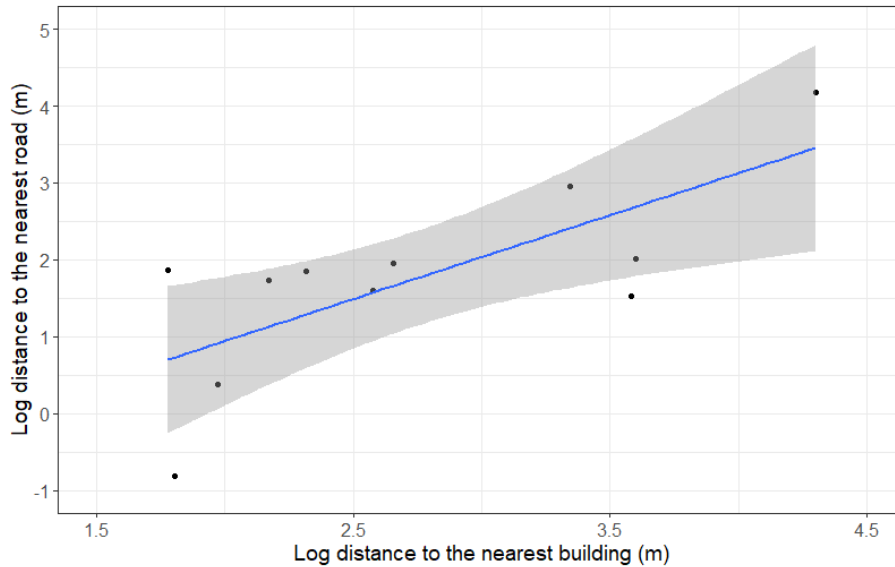


Figure 6: Correlation between the distance to the nearest building and the distance to the nearest road. Simple regression, using a linear model, suggested that the formula of the line was $y = -1.24 + 1.10 x$, the standard error of the slope estimate was 0.33, the p -value was 0.010, and R^2 was 0.54. Pearson's r was 0.73. Both distances were on a natural logarithmic scale and were calculated in QGIS, using spatial data of the Zernike Campus and the coordinates of the nest boxes ($N=11$).

Table 2: Selection of the best model. The average AIC value for the models without the log of the distance to the nearest road was lower, so better. Therefore, this variable was excluded from the models and the log of the distance to the nearest building is maintained. The AIC values were calculated from additive linear mixed-effect models in R. The date, the log of the light intensity, and the log of the distance to either the nearest road or building were fixed effects. The bird and the average daily temperature were random effects.

Model	Onset AIC	Offset AIC	Duration AIC	Average AIC
Excl. distance road	27.00	-70.82	462.49	139.56
Excl. distance building	28.30	-71.25	464.07	140.37

Comparison of logger and telemetry data

Logger and telemetry data for the onset, offset and duration of the active phase were compared in linear models. Furthermore, it was explored how the average daily ambient temperature covaried with the correlation between the logger and telemetry data, by including it as an interaction term in the linear models. For example, a possible explanation for a relatively later logger than telemetry onset is that a bird was already active (telemetry), but waited to leave the nest box because of cold weather. Similarly, the telemetry offset might be relatively later than the logger offset, and the telemetry activity duration might be relatively longer than the logger duration. Again, all models were checked for a normal and homogenous distribution of the residuals by quantile-quantile plots and Shapiro-Wilk tests.

Results

Logger data: effects of light intensity, distance to the nearest building and date

The average light intensity of the occupied nest boxes ($N=11$) was 4.85 lux (range: 0.35 – 17.00 lux). On a natural logarithmic scale, the mean was 0.95 units (range: -1.05 – 2.83 units). The average distance to the nearest buildings was 21.83 m (range: 5.92 – 73.68 m), and on a natural logarithmic scale 2.74 units (range: 1.77 – 4.30 units). See Figure 7 for a map with the locations of the occupied nest boxes with respect to the buildings, roads and measured light intensities. The average onset of activity was 3.92 min after dawn (range: 106.71 min before dawn – 36.94 min after dawn), the average offset was 33.20 min before to dusk (range: 65.53 – 10.48 min before dusk), and the average duration of the active phase was 93.90% of the daylight fraction of the day (range: 84.41 – 109.72 %). The average daylight period during the course of the study period was 10.27 h, which means that the birds were on average 9.64 h active (range: 8.67 – 11.27 h).

Bird activity was well repeatable. The repeatabilities of the onset and duration of the active phase were high (0.77 and 0.78, respectively), and the repeatability of the offset was moderate (0.54). Repeatabilities of the average temperature were poor for all activity measures (<0.50) (*Table 3*). Bird activity was thus consistent between days within individuals, but temperature varied over the recording period (between dates). The latter was previously suggested and shown in *Figure S6*. The within and between individual variation is visualized in *Figure S7*.

ALAN might have affected the timing of activity of the Great tits. The onset of daily activity weakly tended to advance with 3.57 min per unit increase in light intensity ($t_{13.82}=-0.64$, $p=0.54$) (*Figure 8A*). The offset of daily activity delayed to a similar extent. There was a strong trend that the offset became 4.38 min later when the light intensity was stronger ($t_{10.99}=1.94$, $p=0.078$) (*Figure 8B*). The indications of an earlier onset and later offset resulted in a very weak tendency of a 0.72% longer duration of the active phase with increasing light intensities ($t_{5.72}=0.37$, $p=0.73$) (*Figure 8C*). Based on the average daylength of 10.27 h over the 86 days of all activity measurements, this would indicate a 4.42 min longer active period per unit increase in light intensity. Thus, with increasing ALAN, there were indications that the onset advanced, the offset delayed, and the active period became longer.

The distance to the nearest building affected the timing of bird activity to a similar extent as the light intensity. There was a weak indication of an advanced onset of 7.27 min relative to dawn when the roosting site was farther away from the nearest building ($t_{12.80}=-0.92$, $p=0.37$) (*Figure 8D*). This slope was steeper than that of the light intensity, indicating that the advance in onset was stronger for one unit increase in distance than for one unit increase in light intensity. In contrast, the slope of the offset was shallower for the distances than for light. Namely, there was a weak indication of a 2.24 min delay in offset when farther away from the nearest building ($t_{9.57}=0.72$, $p=0.49$) (*Figure 8E*). The relative duration of the active phase weakly tended to become 2.65% (16.32 min) longer with an increasing distance to the nearest building ($t_{5.86}=1.06$, $p=0.33$) (*Figure 8F*), which was more pronounced than for light. So, the farther away from the nearest building, the earlier the onset, the later the offset, and the longer the duration of the active phase.

The timing of activity changed over the course of the study period. The onset strongly tended to advance with 0.26 min relative to dawn per day ($t_{72.53}=-1.42$, $p=0.16$) (*Figure 8G*). Based on the 86 days of activity measurements, the last day would have been 22.36 min relative to dawn earlier than the first day. The offset advanced with 0.24 min relative to dusk per day ($t_{22.95}=-2.53$, $p=0.019$), resulting in 20.64 min over the course of the activity measurements (*Figure 8H*), which is similar to the advance in onset. Surprisingly, despite the advance of both the onset and the offset, there was a strong indication of a 0.075% (0.46 min) longer duration of the active phase per day ($t_{37.96}=1.74$, $p=0.090$), and a 39.56 min longer duration of the active phase at the end of the 86 days (*Figure 8I*). Thus, the onset and offset became each day a little bit earlier, and the duration a little bit longer.

Table 3 shows an overview of the results. Note that most p-values were not-significant (>0.05), standard errors were large, and confidence intervals overlapped with zero. So, there was little confidence of most of the slope estimates. The models for the onset and duration of the active phase did not meet the requirement of a normal distribution of the residuals. As illustrated in *Figure S8*, there was one very early onset and one very long duration. Although these data were biologically relevant, they may have contributed to a deviation from a normal distribution. Therefore, this most extreme left tail outlier was removed from the onset data (early bird), and the most extreme right tail observation was excluded from the duration data (long duration). The models were run again, without these outliers. As shown in *Table S4*, the model parameters of the models with and without the outliers remained similar, indicating that the initial, non-normal models were robust towards the early onset and long duration. Therefore, these first models were kept as the main models.

Possible effects of the parallel running experiment to determine the free running period were not taken into account in the models. Nevertheless, birds returning from constant dim-light conditions may have had different rhythms than birds that were continuously exposed to external cues at the campus. To check if this was the case, bird activity measurements from before and after the experimental dim-light conditions were visually compared (see *Figure S9*). We did not know whether the birds returning from Wageningen were present in our nest boxes, except for one bird (number 12

in box 115, see below). Therefore, we compared the activity timing of all birds before the experiment with all birds after the experiment to get a general idea of a potential effect of the dim-light conditions. Activity timing remained similar, except for one bird (number 14). This bird was earlier in the morning, resulting in a longer total duration of the active phase. Since the first activity measurement of this bird was 9 days after the experiment, and previous research in the lab has shown that initial free running Great tits were entrained to 0.5 lux within 3 days (Spoelstra et al. 2018), it was not assumed that the experiment affected the timing of the measured activity patterns. Even if bird 14 has been in the dim-light conditions, it was probably just an early bird.

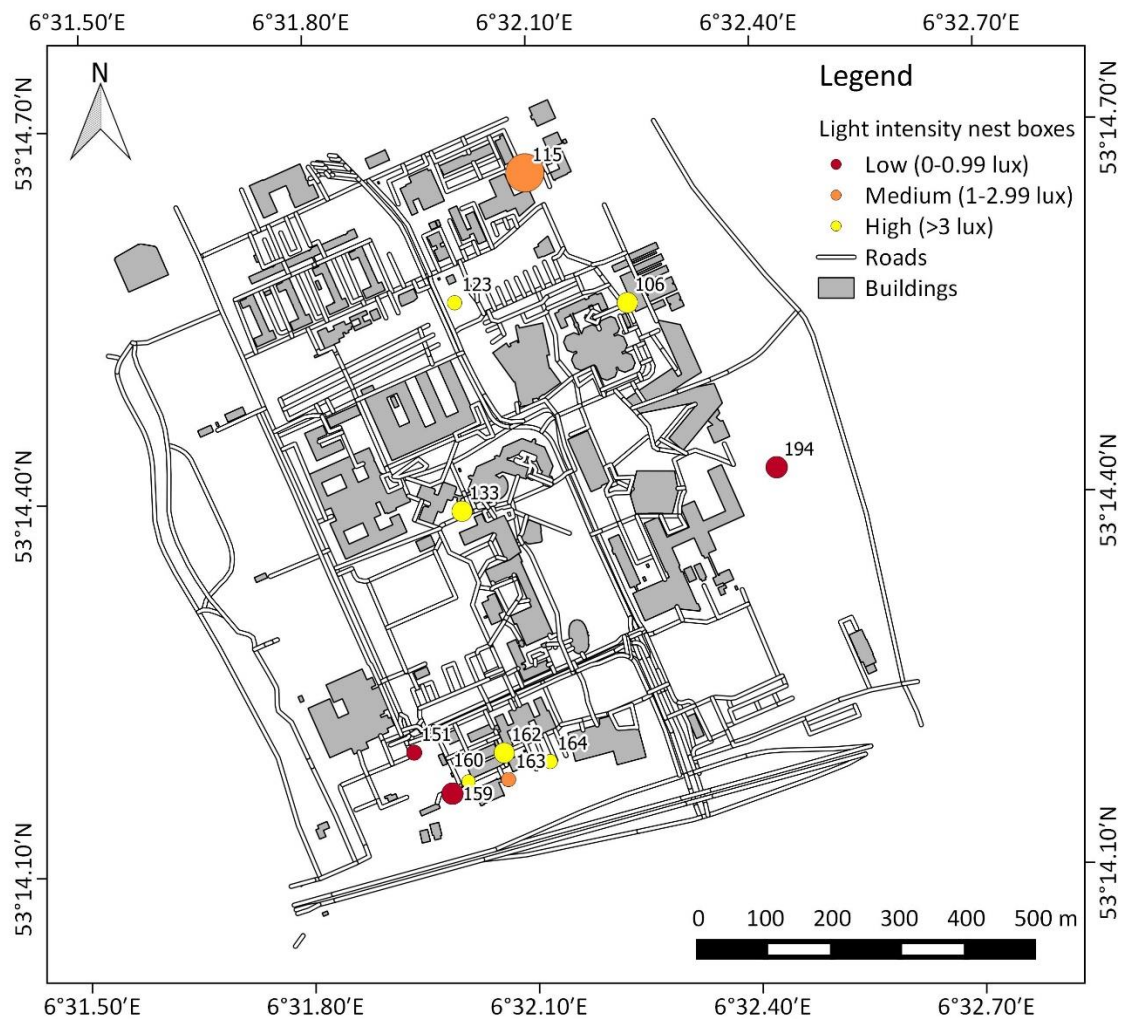


Figure 7: Occupied nest boxes relative to the number of recorded nights, the light intensity, and distances to the buildings and roads. The larger the dot of the nest box, the more often it was occupied during the nights. A red dot represents a nest box that was exposed to a low light intensity (0-0.99 lux), an orange dot represents a medium light intensity (1-2.99 lux) and a yellow dot a high light intensity (>3 lux). Note that the light intensity is categorical here, but treated as a continuous variable in the analyses. The numbers indicate the number codes of the nest boxes. Lines indicate the roads, and grey blocks the buildings. This map was made in QGIS with OpenStreetMap data layers, which were accessed via download.geofabrik.de.

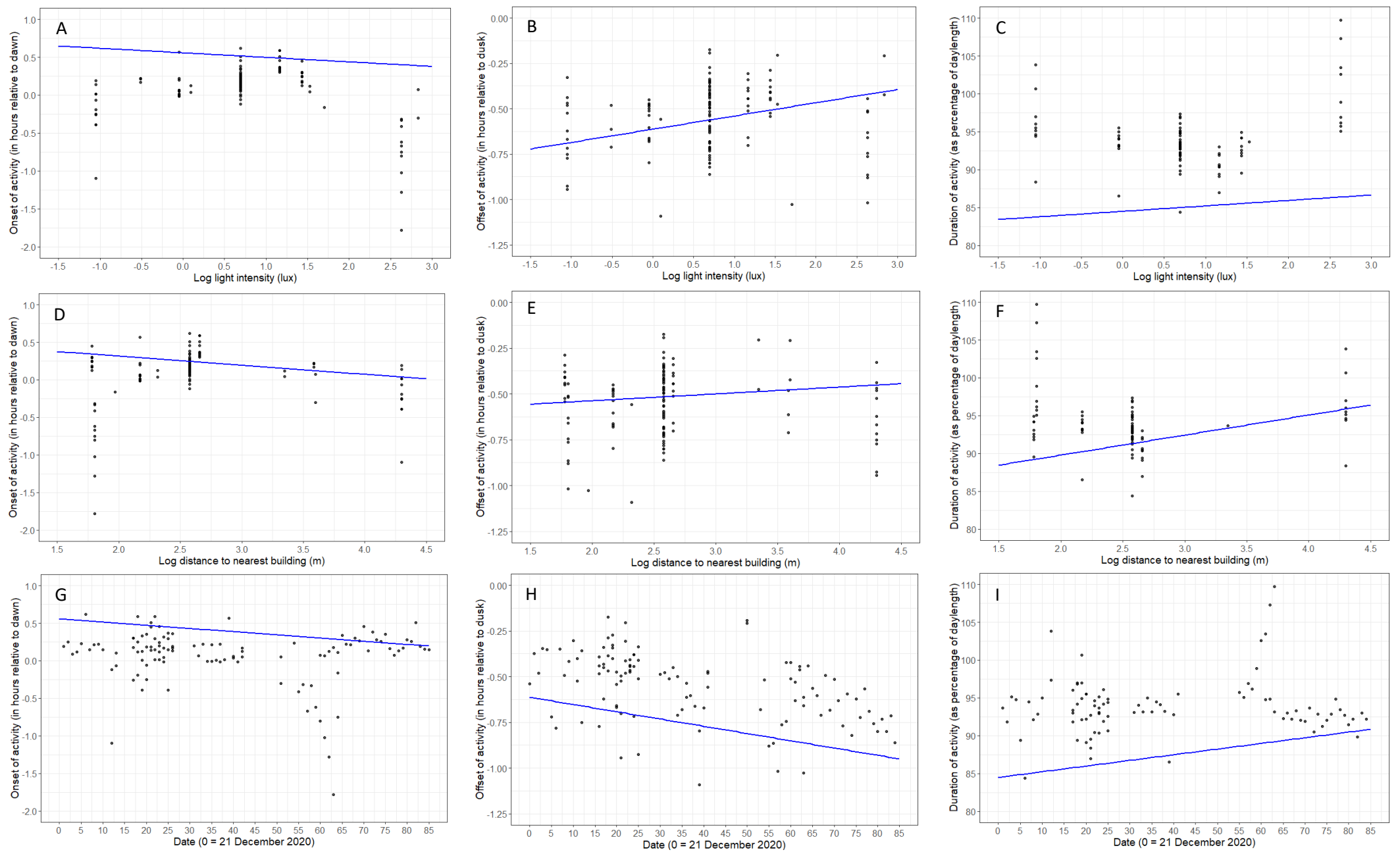


Figure 8: Visualization of the model estimates. With increasing light intensities, the models predicted: A) a weak tendency of an earlier onset of daily activity, B) a strong indication of a delayed offset and C) a very weak tendency of a longer duration of the active phase. When the distance to the nearest building increased, the models predicted weak indications of: D) an advanced onset, E) a later offset, and F) a longer duration of the active phase. Over the course of the study period, the models predicted: G) a weak tendency of earlier onsets, H) later offsets, and I) a strong indication of longer durations of the active phase. Model parameters were based on additive linear mixed-effect models with average temperature and bird as random effects and the light intensity, the distance to the nearest building and date as fixed effects. The back dots are the observations, and the blue lines have the function $y=ax+b$, where “a” is the predicted slope of the variable and “b” the model intercept. The light intensity and distance to the nearest building were on a natural logarithmic scale. The date, which ranged from 21 December 2020 to 16 March 2021, was on a numeric scale, where date 0 indicated 21 December 2020. The onset and offset were expressed in decimal hours relative to dawn and dusk, where a negative value means before dawn/dusk and a positive value after dawn/dusk. The duration was expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset).

Table 3: Overview of model parameters for the onset, offset and duration of the active phase. Slope estimates are expressed in hours relative to dawn for the onset, to dusk for the offset, and percentages of the daylight fraction of the day for the duration. Slope estimates between parentheses indicate the non-log estimates in minutes (unless otherwise indicated). Models were additive linear mixed-effects models, run with the lme4 package in R. Fixed effects were the light intensity (lux), the distance to the buildings (m) and the date (numeric, day 0 = 21 Dec 2020). Light intensity and distance to the nearest building were on natural logarithmic scales. Bird identity and average temperature were added as random effects. “r bird” and “r temp” indicate the repeatabilities (ICCs) for the bird number and the average temperature, respectively.

Model	Variable	Estimate	SE	df	t-value	Pr(> t)	2.5 – 97.5% CI	r bird	r temp
Onset	(Intercept)	0.56 (33.41)	0.44	14.09	1.25	0.23	-0.27 – 1.38	0.77	0.23
	Light intensity (log)	-0.060 (-3.57)	0.094	13.82	-0.64	0.54	-0.23 – 0.11		
	Dist. building (log)	-0.12 (-7.27)	0.13	12.80	-0.92	0.37	-0.36 – 0.12		
	Date	-4.25*10 ⁻³ (-0.26)	2.99*10 ⁻³	72.53	-1.42	0.16	-9.94*10 ⁻³ – 1.63*10 ⁻³		
Offset	(Intercept)	-0.61 (-36.76)	0.18	10.01	-3.44	6.34*10 ⁻³	-0.94 – -0.28	0.54	0.46
	Light intensity (log)	0.073 (4.38)	0.038	10.99	1.94	0.078	5.06*10 ⁻³ – 0.14		
	Dist. Building (log)	0.037 (2.24)	0.052	9.57	0.72	0.49	-0.057 – 0.13		
	Date	-3.97*10 ⁻³ (-0.24)	1.57*10 ⁻³	22.95	-2.53	0.019	-6.68*10 ⁻³ – -1.11*10 ⁻³		
Duration	(Intercept)	84.48 (8.67 h)	8.33	5.97	10.13	5.55*10 ⁻⁵	70.13 – 98.90	0.78	0.38
	Light intensity (log)	0.72 (4.42)	1.94	5.72	0.37	0.73	-2.63 – 4.08		
	Dist. Building (log)	2.65 (16.32)	2.49	5.86	1.06	0.33	-1.65 – 6.94		
	Date	0.075 (0.46)	0.043	37.96	1.74	0.090	-0.011 – 0.15		

Comparison of logger and telemetry data

Logger and telemetry data could be compared for one bird (number 12 in box 115) between 20 February and 2 March 2021. The slopes of the regression between the telemetry (predictor variable) and logger data (response variable) were for all activity measures smaller than 1. When the telemetry onset increased by 1 h, the logger onset increased by 0.53 h ($F_{1,9}=14.75$, $R^2=0.62$, $p=0.0040$), and when the telemetry duration increased by 1%, the logger duration increased by 0.69% ($F_{1,7}=5.80$, $R^2=0.45$, $p=0.047$). There was a trend of the logger offset increasing by 0.77 h when the telemetry data increased by 1 h ($F_{1,7}=3.06$, $R^2=0.30$, $p=0.12$). See *Figure 9* and *Table S5* for an overview of these results. The slope estimates were much smaller when the telemetry data were not corrected by 1 h (*Table S6*), indicating that the shifted data were indeed a mistake during data processing.

Radio telemetry measured an earlier onset, an on average a slightly later offset, and a longer duration of the active phase than loggers. The telemetry onset was on average 16.20 min earlier than the logger onset (range: 6.35 – 28.58 min earlier), the offset was on average 0.92 min later (range: 7.90 min earlier – 9.70 min later), and the duration was on average 2.67% longer (range: 1.41 – 4.12% longer). Given the average daylength of 11.73 h during the period of logger and telemetry comparison, the duration of the active phase from the telemetry data would have been on average 31.36 min longer than the duration from the logger data (range: 16.54 – 48.33 min longer). See *Table 4* and also *Table S3* for overviews of the telemetry and logger data. When we checked whether the relationship between the two activity measures depended on the average ambient temperature, we found no clear patterns (for details see *Table S5*).

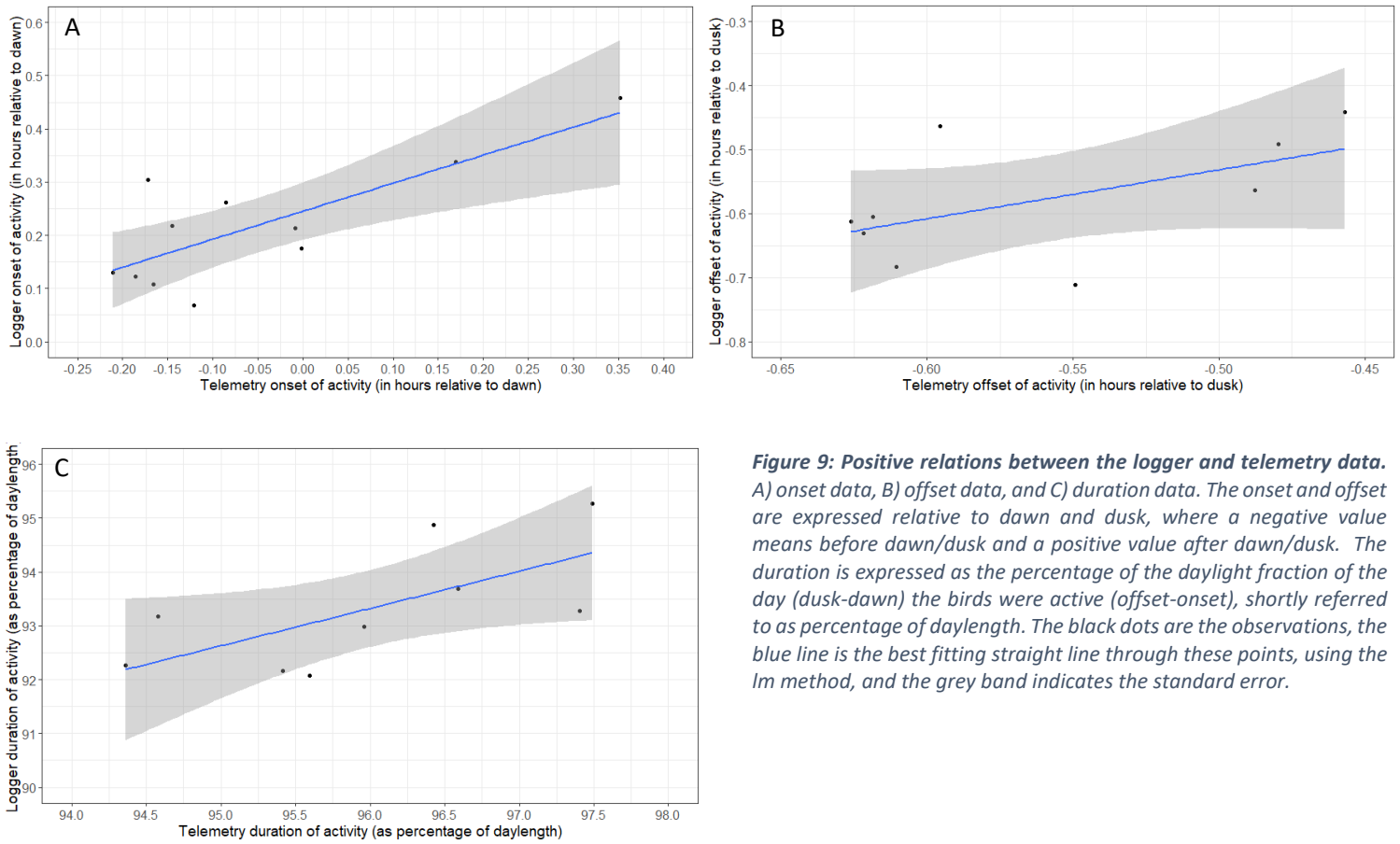


Figure 9: Positive relations between the logger and telemetry data. A) onset data, B) offset data, and C) duration data. The onset and offset are expressed relative to dawn and dusk, where a negative value means before dawn/dusk and a positive value after dawn/dusk. The duration is expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset), shortly referred to as percentage of daylength. The black dots are the observations, the blue line is the best fitting straight line through these points, using the lm method, and the grey band indicates the standard error.

Table 4: Overview of the average, minimum and maximum times of onsets and offsets, and of durations. Telemetry onsets were earlier than the logger data, telemetry offsets were on average later, and durations longer. A “T” indicates telemetry and a “L” indicates logger. The onset and offset are shown as time in h:m:s format. The duration is shown as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset).

Value	Onset T (hms)	Onset L (hms)	Offset T (hms)	Offset L (hms)	Duration T (%)	Duration L (%)
Average	06:53:27	07:09:39	18:06:40	18:06:11	95.98	93.31
Minimum	06:33:00	06:53:28	17:57:00	17:57:49	94.36	92.07
Maximum	07:09:00	07:19:07	18:15:00	18:16:32	97.49	95.27

Discussion

A shortcoming of previous studies about the effects of ALAN on activity patterns of birds was the shortage of data outside the breeding season, and the absence of studies that related this timing to the spatial habitat. Therefore, the current study focused on the timing of Great tit activity during winter, and related this timing besides ALAN also to the distance to the nearest building. I found indications of an earlier onset, a later offset and a longer duration of the active phase with increasing light intensities. Similar results were found with increasing distances to the nearest building. So, this pilot study showed that both ALAN and the spatial habitat may shift the timing of Great tit activity. We used a novel, non invasive technique to measure bird activity, which was based on passive infrared loggers that detect bird movements inside nest boxes. Logger data were compared with data from conventional telemetry techniques, and found to be comparable, but a bit later in the morning, on average slightly earlier the evening, and shorter in the duration than telemetry data. These loggers are therefore promising for future studies.

It was hypothesized that birds roosting in a nest box with a higher light intensity have an earlier onset, a later or unchanged offset, and a longer duration of the active phase. These hypotheses were supported by our results. The observed advance in morning activity onset with increasing light intensities supports existing evidence from the literature (Kempenaers et al. 2010, Dominoni et al. 2014, 2020, de Jong et al. 2016, 2017, Spoelstra et al. 2018, Bermúdez-Cuamatzin et al. 2020). However, our tendency and effect of a delayed activity offset is not consistently shown in previous research. Most studies reported a smaller effect on the offset than on the onset (Da Silva et al. 2014, de Jong et al. 2017, Dominoni et al. 2020), or even no effect on the offset (Dominoni et al. 2014). In contrast, the current study found that the delay in offset covered a similar, and even slightly larger amount of minutes as the advance in onset (4.38 and 3.57 min, respectively). The effect on the onset is consistent with previous findings on Great tits outside the breeding season, but the effect on the offset is not (Raap et al. 2015). An explanation for the difference with our results is the lighting method. Raap et al. (2015) illuminated nest boxes from the inside, so the environment outside the nest boxes was not substantially illuminated. Hence, their light did not allow the birds to extend their activity in the evening, unlike the light in our study.

A potential physiological mechanism underlying an advanced onset and delayed offset due to ALAN involves the hormone melatonin. Melatonin enables synchronization of the circadian clock to the light-dark cycle, and regulates the physiological detection of daylength (Pfeffer et al. 2018). Melatonin secretion is suppressed by light, which may in turn lead to a prolonged perceived daylength and changed activity rhythms (Dominoni et al. 2013). We do not know, however, why our results show a relatively large effect on the offset compared to other studies. An interesting angle for further research is to investigate whether the duration of melatonin suppression contributes to the extent of the shift in onset and offset of activity.

Although we found a similar extent of the delay in offset and the advance in onset, light might still have been a stronger cue for activity onset than offset. De Jong et al. (2016) reported a clear coincidence of activity onset, but not offset, with the time when light was switched on or off in an experimental study on Great tits. Consequently, there was a larger variability in activity offset than onset. Although we do not have data of the time when the street lights turned on or off, we did see that the repeatability of the offset was lower than of the onset. This indicates that also here was a larger variability in activity offset, meaning that light might have been more important for the timing of the onset than the offset.

The indication of a longer duration of the active phase with increasing light intensities logically followed from the observed earlier onset and later offset, which is in line with the results from De Jong et al. (2016, 2017). However, it is important to realize that this does not directly mean that the birds were more active. Our definition of duration was based on the difference between the offset and onset time, instead of real continuous measurements of bird activity. For instance, Bermúdez-Cuamatzin et al. (2020) compared the singing activity of urban and rural birds, and found that urban birds sang earlier in the morning than rural birds, but less during the rest of the day. The latter effect would not have been found if they had subtracted the time of the first song from that of the last song. Similarly,

De Jong et al. (2016, 2017) reported that the total amount of activity in response to ALAN did not change: the birds spread their activity over the active phase. Dominoni et al. (2020), in contrast, showed that Great tits exposed to 5 lux in the laboratory were more active over the whole 24 h period. So, although the duration of the active phase was found to be longer, it is not completely sure whether this indicates a larger total amount of activity or not.

With increasing distances to the nearest building, there were indications of an earlier onset, a later offset, and a longer duration of the active phase of Great tit activity. We do not know the meaning of these effects yet. It might be that the distance to the buildings was confounded by ALAN: the farther away from the building, the closer to a street light. However, there was no strong correlation between the light intensity and the distance to the nearest building (Pearson's r was -0.39), and we selected our nest box locations carefully with respect to light intensities and urban characteristics. One could also reason that birds closer to buildings can afford to be later, due to ad libitum access to bird feeders or anthropogenic food items. Birds farther away from the buildings, on the other hand, have to work harder for their food, and rising early or sleeping late might be a strategy to outcompete others. However, this hypothesis might not hold for such a small scale as the Zernike Campus. Furthermore, research has shown that birds in a better body condition and/or higher social status (e.g. due to a territory with a bird feeder) may sing earlier (Poesel et al. 2006, Murphy et al. 2008), which may contrast with the competition hypothesis (although they are not mutually exclusive). Namely, the competition hypothesis assumes that birds farther away from buildings (and food) may be earlier, whereas the body condition hypothesis assumes that birds closer to buildings (and food) may be earlier. The latter hypothesis could in turn also point into an indirect effect of ALAN on the activity timing, because streetlights are known to attract many insects (Davies et al. 2012). Buildings (and roads, to which buildings were correlated) may also indicate noise. Research has shown that also noise can advance bird activity (Dominoni 2020), but under this assumption our results point into the opposite direction. Note that examining this so-called multisensory pollution (Dominoni 2020) was beyond the scope and aim of this study, and that the campus was also less crowded and disturbed as usual due to the pandemic restrictions. So, although we do not know the reasons for the effects of the spatial habitat on the timing of bird activity, it opens interesting avenues for further research.

The onset and offset of activity changed relative to dawn and dusk over the course of the study period, which is in line with the findings of Da Silva et al. (2014). Surprisingly, the duration became longer over the course of the study period, whereas both onset and offset times advanced. The exact reason remains unknown, but it may be due to the gap of duration data after the first half of the study period. However, the gap in the duration data was also present for the effects of the distance to the buildings and the light intensity, and the duration changed logically for those variables.

Loggers gave later onsets, on average slightly earlier offsets, and longer durations than telemetry did, which can be explained by the ways in which these two different methods led to the activity measures. Telemetry detected the onset as the change in signal strength when a bird started to be active, which required movement of the bird. Loggers detected the onset as the time just before the bird left the nest box in the morning (vice versa for the offset). An earlier telemetry than logger onset may have logically resulted from birds already moving in a nest box before they left. However, we are not sure if the movements in such a small space as a nest box were sufficient to cause a change in signal strength. Another possible reason is that a later logger onset resulted from birds leaving the box a few times before properly starting the day. Similarly, a later telemetry offset may have resulted from birds moving inside a nest box after they came back in the evening, or birds that flew a few times in and out before properly ending their day. Interestingly, we have also found telemetry offsets that were earlier than logger offsets. This remains an unexplained finding for now, but we will further investigate this. Lastly, it should be noted that differences smaller than 3 min need to be interpreted with care. Telemetry data was binned in 3-min intervals, meaning that all onset and offset times of the telemetry data were the earliest timepoint in such an interval. It is therefore hard to tell if onset and offset times of the logger data, that fitted within the telemetry interval, are really different from the onset and offset times of the telemetry data. Nevertheless, our loggers have proven to be a new, non-invasive technique to measure bird activity, and therefore have promising potential for future studies.

The generalizability of the results is limited by a small number of birds and sometimes a small number of activity measurements per bird, confidence intervals that overlapped with zero, and large p-values and standard errors. Furthermore, I acknowledge that the birds were free to choose the nest boxes based on the light intensity and other factors. The birds may have selected the boxes that fitted their quality and potentially their chronotype. Birds could also fly around the campus and were therefore probably exposed to various light intensities. Also the loggers themselves may have affected the nest box choice and activity patterns of the birds. Namely, a 0.4 lux red light turned for 5 sec when the logger detected movement. Although it was not assumed that red light changed rhythms or increased night time activity (Ouyang et al. 2017), it might have been scary, forcing some birds to find a different box. We have recently developed loggers without this light, so this will not be an issue for future research.

A final point of discussion is the light intensity measurements. As previously mentioned, not all light measurements could be done on the same day. Thereby, the light intensity was not repeatedly measured over the course of the study period, because of restrictions by a Covid-19 curfew. Certain bright lights at the sports centre were dimmed, due to a temporary roof over the tennis court. Also lights of ongoing construction work south-east and south-west of the Linnaeusborg may have altered the light intensity around the nest boxes. These changes in light, that could have been perceived by the birds, could not be measured by us. Furthermore, we noticed that some streetlights switched on and others off during our measurements, which could have led to under- and overestimations of the light intensity. A better collaboration with the light management of the campus would solve this problem. I would also recommend repeated measures, or even continuous light measurements in further studies at the Zernike Campus.

Nonetheless, our results emphasized that ALAN can affect activity rhythms, and indicated the potential to studying these rhythms with respect to the spatial habitat. While previous research has focused on the effects of ALAN during breeding, our results indicate that these effects persist into roosting behaviour during the non-breeding season. ALAN is known to affect many circadian and circannual rhythms, with impacts translating into the ecosystem and fitness level (Sanders et al. 2020). Therefore, it is likely that besides behavioural rhythms also other rhythms were disturbed in our birds, with possible negative consequences for health and fitness. For instance, wild Great tits with elevated night-time activity levels due to ALAN, also had physiological markers of sleep debt and impaired disease dynamics (Ouyang et al. 2017). Physiological disruption was also shown in the lab: ALAN led to a shift in activity rhythms, which was followed by only a minority of the analysed genes and metabolites, pointing to internal desynchronisation (Dominoni et al. 2020). Furthermore, a recent study reported that a higher light sensitivity and intensity advanced reproductive timing (Senzaki et al. 2020), potentially leading to phenological mismatches (Visser and Gienapp 2019). So, the observed change in activity rhythms may be only a small piece of what is actually going on, not only in Great tits, but also in other organisms. More research is needed to better understand the detrimental effects of ALAN, and its implications for health, fitness, and ecosystem functioning.

This pilot study was one of the first in a series of studies on this population at the Zernike Campus. During the subsequent breeding season, almost half of our nest boxes was occupied and about 300 eggs were laid. The chicks were ringed, measured and weighted before fledging, which provided a solid basis for a from now on closely monitored population. My research, as part of a long project about the effects of ALAN on Great tits, will contribute to a better understanding of sensory, spatial, and urban ecology, and it will open new doors to conserve urban biodiversity. More knowledge is really needed, because the urbanised landscape is one of the leading causes of local biodiversity loss (McKinney 2002). Light pollution is considered as a biodiversity threat (Hölker et al. 2010b), and continues to increase by about 6% per year (Hölker et al. 2010a). There is thus a growing need for studies like ours. Insights from these studies will help to mitigate the negative effects of ALAN and promote the development of biodiversity friendly cities throughout the world. More locally, the results can have a great value in making the campus more innovative, sustainable and nature friendly. The reason is simple: in order to see the light of nature, darkness must be present.

References

- Bennie, J., T. W. Davies, D. Cruse, and K. J. Gaston. 2016. Ecological effects of artificial light at night on wild plants. *Journal of Ecology* 104:611–620.
- Bermúdez-Cuamatzin, E., Z. Delamore, L. Verbeek, C. Kremer, and H. Slabbekoorn. 2020. Variation in Diurnal Patterns of Singing Activity Between Urban and Rural Great Tits. *Frontiers in Ecology and Evolution* 8:246.
- Chen, Y.-R., W.-L. Wei, D. T. W. Tzeng, A. C. S. Owens, H.-C. Tang, C.-S. Wu, S.-S. Lin, S. Zhong, and E.-C. Yang. 2021. Effects of artificial light at night (ALAN) on gene expression of *Aquatica ficta* firefly larvae. *Environmental Pollution* 281:116944.
- Da Silva, A., J. M. Samplonius, E. Schlicht, M. Valcu, and B. Kempenaers. 2014. Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behavioral Ecology* 25:1037–1047.
- Davies, T. W., J. Bennie, and K. J. Gaston. 2012. Street lighting changes the composition of invertebrate communities. *Biology Letters* 8:764–767.
- Dominoni, D. 2020. Multisensory pollution: Artificial light at night and anthropogenic noise have interactive effects on activity patterns of great tits (*Parus major*). *Environmental Pollution* 256:113314.
- Dominoni, D. M., E. O. Carmona-Wagner, M. Hofmann, B. Kranstauber, and J. Partecke. 2014. Individual-based measurements of light intensity provide new insights into the effects of artificial light at night on daily rhythms of urban-dwelling songbirds. *Journal of Animal Ecology* 83:681–692.
- Dominoni, D. M., W. Goymann, B. Helm, and J. Partecke. 2013. Urban-like night illumination reduces melatonin release in European blackbirds (*Turdus merula*): implications of city life for biological time-keeping of songbirds. *Frontiers in Zoology* 10:60.
- Dominoni, D. M., M. de Jong, K. van Oers, P. O’Shaughnessy, G. Blackburn, E. Atema, C. A. Mateman, P. B. D’Amelio, L. Trost, M. Bellingham, J. Clark, M. E. Visser, and B. Helm. 2020. Artificial light at night shifts the circadian system but still leads to physiological disruption in a wild bird. *bioRxiv:2020.12.18.423473*.
- Dwyer, R. G., S. Bearhop, H. A. Campbell, and D. M. Bryant. 2013. Shedding light on light: benefits of anthropogenic illumination to a nocturnally foraging shorebird. *The Journal of Animal Ecology* 82:478–485.
- Ebbensgaard, C. 2015. Urban Lighting, Light Pollution and Society. *European Planning Studies* 23:1437–1440.
- Falchi, F., P. Cinzano, D. Duriscoe, C. C. M. Kyba, C. D. Elvidge, K. Baugh, B. A. Portnov, N. A. Rybnikova, and R. Furgoni. 2016. The new world atlas of artificial night sky brightness. *Science Advances* 2:e1600377.
- Falcón, J., A. Torriglia, D. Attia, F. Viénot, C. Gronfier, F. Behar-Cohen, C. Martinsons, and D. Hicks. 2020. Exposure to Artificial Light at Night and the Consequences for Flora, Fauna, and Ecosystems. *Frontiers in Neuroscience* 14:602796.
- Foster, R. G., and L. Kreitzman. 2005. *Rhythms of Life: The Biological Clocks that Control the Daily Lives of Every Living Thing*. Yale University Press, London, England.
- Gaston, K. J., J. P. Duffy, S. Gaston, J. Bennie, and T. W. Davies. 2014a. Human alteration of natural light cycles: causes and ecological consequences. *Oecologia* 176:917–931.
- Gaston, K. J., S. Gaston, J. Bennie, and J. Hopkins. 2014b. Benefits and costs of artificial nighttime lighting of the environment. *Environmental Reviews* 23:14–23.
- Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: the research challenge. *Philosophical Transactions of the Royal Society B: Biological Sciences* 370:20140133.
- Gurarie, E., R. D. Andrews, and K. L. Laidre. 2009. A novel method for identifying behavioural changes in animal movement data. *Ecology Letters* 12:395–408.
- van Hasselt, S. J., R. A. Hut, G. Allocca, A. L. Vyssotski, T. Piersma, N. C. Rattenborg, and P. Meerlo. 2021. Cloud cover amplifies the sleep-suppressing effect of artificial light at night in geese. *Environmental Pollution* 273:116444.
- Helm, B., M. E. Visser, W. Schwartz, N. Kronfeld-Schor, M. Gerkema, T. Piersma, and G. Bloch. 2017. Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372:20160246.

- Hölker, F., T. Moss, B. Griefahn, W. Kloas, C. C. Voigt, D. Henckel, A. Hänel, P. M. Kappeler, S. Völker, A. Schwoppe, S. Franke, D. Uhrlandt, J. Fischer, R. Klenke, C. Wolter, and K. Tockner. 2010a. The Dark Side of Light: A Transdisciplinary Research Agenda for Light Pollution Policy. *Ecology and Society* 15:art13.
- Hölker, F., C. Wolter, E. K. Perkin, and K. Tockner. 2010b. Light pollution as a biodiversity threat. *Trends in Ecology & Evolution* 25:681–682.
- Hoyo, J. del, J. Sargatal, and J. Cabot. 1992. *Handbook of the Birds of the World*. Lynx Edicions, Barcelona, Spain.
- Hughes, T. P. 2004. *American Genesis: A Century of Invention and Technological Enthusiasm, 1870-1970*. University of Chicago Press, Chicago, Illinois, United States.
- Jarrett, C., L. L. Powell, H. McDevitt, B. Helm, and A. J. Welch. 2020. Bitter fruits of hard labour: diet metabarcoding and telemetry reveal that urban songbirds travel further for lower-quality food. *Oecologia* 193:377–388.
- Jonasson, K. A. 2017. *The Effects of Sex, Energy, and Environmental Conditions on the Movement Ecology of Migratory Bats*. Electronic Thesis and Dissertation Repository 4411.
- de Jong, M., S. P. Caro, P. Gienapp, K. Spoelstra, and M. E. Visser. 2017. Early Birds by Light at Night: Effects of Light Color and Intensity on Daily Activity Patterns in Blue Tits. *Journal of Biological Rhythms* 32:323–333.
- de Jong, M., L. Jeninga, J. Q. Ouyang, K. van Oers, K. Spoelstra, and M. E. Visser. 2016. Dose-dependent responses of avian daily rhythms to artificial light at night. *Physiology & Behavior* 155:172–179.
- Kempenaers, B., P. Borgström, P. Loës, E. Schlicht, and M. Valcu. 2010. Artificial Night Lighting Affects Dawn Song, Extra-Pair Siring Success, and Lay Date in Songbirds. *Current Biology* 20:1735–1739.
- Koo, T. K., and M. Y. Li. 2016. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine* 15:155–163.
- Krištín, A., I. Mihál, and P. Urban. 2001. Roosting of the great tit, *Parus major* and the nuthatch, *Sitta europaea* in nest boxes in an oak-hornbeam forest. *Folia Zoologica -Praha-* 50:43–53.
- Kvist, L., J. Martens, H. Higuchi, A. A. Nazarenko, O. P. Valchuk, and M. Orell. 2003. Evolution and genetic structure of the great tit (*Parus major*) complex. *Proceedings of the Royal Society B: Biological Sciences* 270:1447–1454.
- Laine, V. N., T. I. Gossmann, K. M. Schachtschneider, C. J. Garroway, O. Madsen, K. J. F. Verhoeven, V. de Jager, H.-J. Megens, W. C. Warren, P. Minx, R. P. M. A. Crooijmans, P. Corcoran, B. C. Sheldon, J. Slate, K. Zeng, K. van Oers, M. E. Visser, and M. A. M. Groenen. 2016. Evolutionary signals of selection on cognition from the great tit genome and methylome. *Nature Communications* 7:10474.
- Lebbin, D. J., M. G. Harvey, T. C. Lenz, M. J. Andersen, and J. M. Ellis. 2007. Nocturnal Migrants Foraging at Night by Artificial Light. *The Wilson Journal of Ornithology* 119:506–508.
- Lövy, M., J. Šklíba, and R. Šumbera. 2013. Spatial and Temporal Activity Patterns of the Free-Living Giant Mole-Rat (*Fukomys mechowii*), the Largest Social Bathyergid. *PLoS ONE* 8:1–9.
- McKinney, M. L. 2002. Urbanization, Biodiversity, and Conservation. *BioScience* 52:883–890.
- Moreno, C. R. C., K. Wright, D. J. Skene, and F. M. Louzada. 2020. Phenotypic plasticity of circadian entrainment under a range of light conditions. *Neurobiology of Sleep and Circadian Rhythms* 9:100055.
- Murphy, M. T., K. Sexton, A. C. Dolan, and L. J. Redmond. 2008. Dawn song of the eastern kingbird: an honest signal of male quality? *Animal Behaviour* 75:1075–1084.
- Nord, A., M. Lehmann, R. MacLeod, D. J. McCafferty, R. G. Nager, J.-Å. Nilsson, and B. Helm. 2016. Evaluation of two methods for minimally invasive peripheral body temperature measurement in birds. *Journal of Avian Biology* 47:417–427.
- Ouyang, J. Q., M. de Jong, R. H. A. van Grunsven, K. D. Matson, M. F. Haussmann, P. Meerlo, M. E. Visser, and K. Spoelstra. 2017. Restless roosts: Light pollution affects behavior, sleep, and physiology in a free-living songbird. *Global Change Biology* 23:4987–4994.
- Pfeffer, M., H.-W. Korf, and H. Wicht. 2018. Synchronizing effects of melatonin on diurnal and circadian rhythms. *General and Comparative Endocrinology* 258:215–221.

- Poesel, A., H. P. Kunc, K. Foerster, A. Johnsen, and B. Kempenaers. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. *Animal Behaviour* 72:531–538.
- Pugh, A., S. Pawson, S. Pawson, and S. Pawson. 2016. Artificial light at night potentially alters feeding behaviour of the native southern black-backed gull (*Larus dominicanus*). *Notornis* 63:37–39.
- Raap, T., R. Pinxten, and M. Eens. 2015. Light pollution disrupts sleep in free-living animals. *Scientific Reports* 5:13557.
- Rich, C., and T. Longcore. 2006. *Ecological Consequences of Artificial Night Lighting*. Page BiblioVault OAI Repository, the University of Chicago Press. Island Press, Washington, D.C.
- Sánchez de Miguel, A., M. Aubé, J. Zamorano, M. Kocifaj, J. Roby, and C. Tapia. 2017. Sky Quality Meter measurements in a colour-changing world. *Monthly Notices of the Royal Astronomical Society* 467:2966–2979.
- Sanders, D., E. Frago, R. Kehoe, C. Patterson, and K. J. Gaston. 2020. A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution* 5:74–81.
- Schofield, L. N., J. L. Deppe, T. J. Zenzal, M. P. Ward, R. H. Diehl, R. T. Bolus, and F. R. Moore. 2018. Using automated radio telemetry to quantify activity patterns of songbirds during stopover. *The Auk* 135:949–963.
- Senzaki, M., J. R. Barber, J. N. Phillips, N. H. Carter, C. B. Cooper, M. A. Ditmer, K. M. Fristrup, C. J. W. McClure, D. J. Mennitt, L. P. Tyrrell, J. Vukomanovic, A. A. Wilson, and C. D. Francis. 2020. Sensory pollutants alter bird phenology and fitness across a continent. *Nature* 587:605–609.
- Spoelstra, K., I. Verhagen, D. Meijer, and M. E. Visser. 2018. Artificial light at night shifts daily activity patterns but not the internal clock in the great tit (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences* 285:20172751.
- Sun, J., T. Raap, R. Pinxten, and M. Eens. 2017. Artificial light at night affects sleep behaviour differently in two closely related songbird species. *Environmental Pollution (Barking, Essex: 1987)* 231:882–889.
- Truscott, Z., D. T. Booth, C. J. Limpus, Z. Truscott, D. T. Booth, and C. J. Limpus. 2017. The effect of on-shore light pollution on sea-turtle hatchlings commencing their off-shore swim. *Wildlife Research* 44:127–134.
- Ulgezen, Z., T. Kämpylä, P. Meerlo, K. Spoelstra, M. Visser, and D. Dominoni. 2019. The preference and costs of sleeping under light at night in forest and urban great tits. *Proceedings of the Royal Society B: Biological Sciences* 286:20190872.
- Van Doren, B. M., K. G. Horton, A. M. Dokter, H. Klinck, S. B. Elbin, and A. Farnsworth. 2017. High-intensity urban light installation dramatically alters nocturnal bird migration. *Proceedings of the National Academy of Sciences of the United States of America* 114:11175–11180.
- Vetter, C. 2020. Circadian disruption: What do we actually mean? *The European Journal of Neuroscience* 51:531–550.
- Visser, M. E., and P. Gienapp. 2019. Evolutionary and demographic consequences of phenological mismatches. *Nature Ecology & Evolution* 3:879–885.
- Wasserstein, R. L., and N. A. Lazar. 2016. The ASA Statement on p-Values: Context, Process, and Purpose. *The American Statistician* 70:129–133.
- Wilson, A. A., M. A. Ditmer, J. R. Barber, N. H. Carter, E. T. Miller, L. P. Tyrrell, and C. D. Francis. 2021. Artificial night light and anthropogenic noise interact to influence bird abundance over a continental scale. *Global Change Biology*:1–18.

Supplement

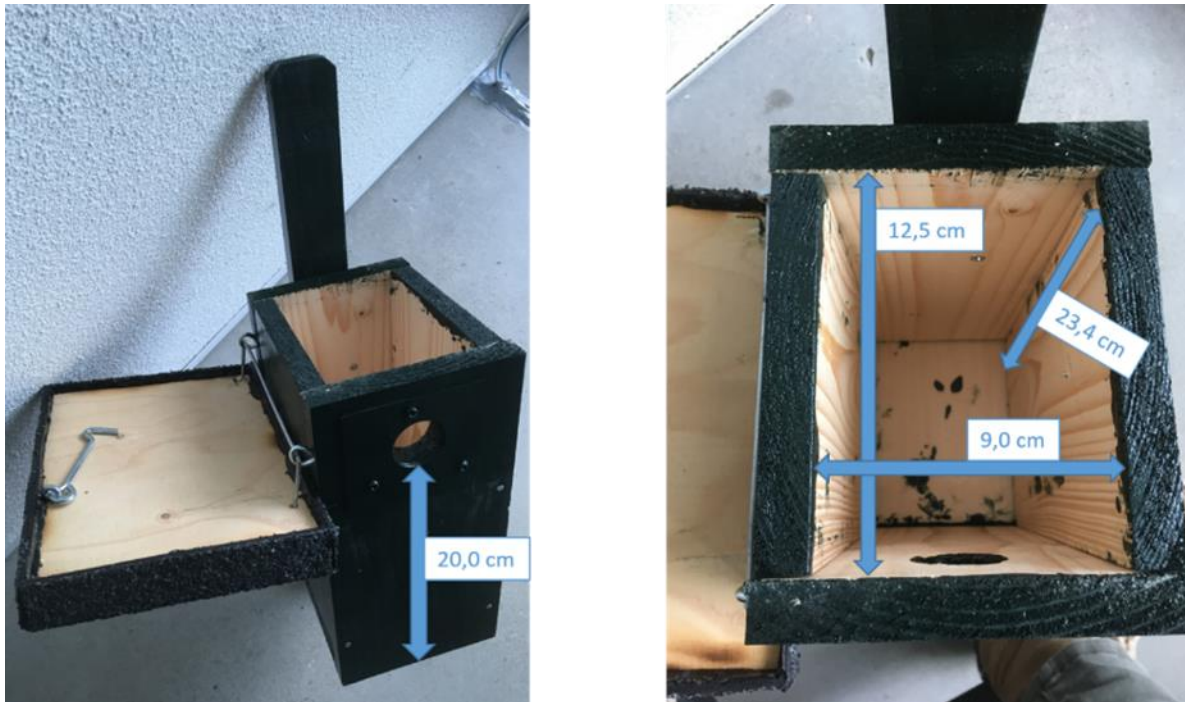


Figure S1: Dimensions of a nest box. The distance from the bottom to the entrance hole is 20.0 cm. The dimensions of the inside of a box are 9.0*12.5*23.4 cm. Not shown: the diameter of the entrance hole is 2.80 cm. Pictures and measurements were made by masters student Nivard Boersma.

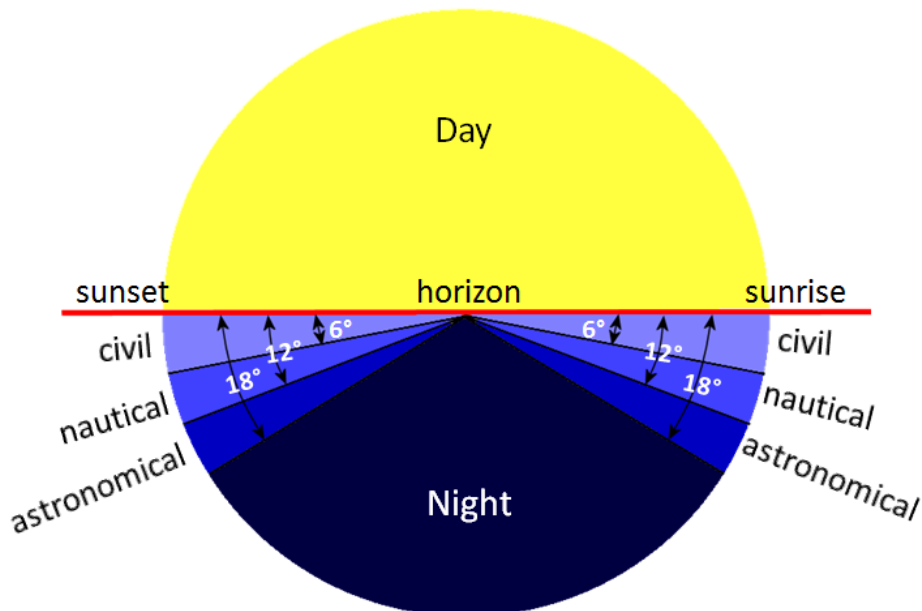


Figure S2: Visualization of the different types of dawn and dusk. Morning civil twilight ends when the sun rises, and evening civil starts when the sun sets. Morning nautical twilight ends, and evening nautical twilight starts when the sun is 6° degrees below the horizon. Morning astronomical twilight ends, and evening astronomical twilights starts when the sun is 12° below the horizon. Astronomical darkness is defined as the period when the sun is >18° below the horizon. Image is adapted from <https://www.weather.gov/fds/twilight>.

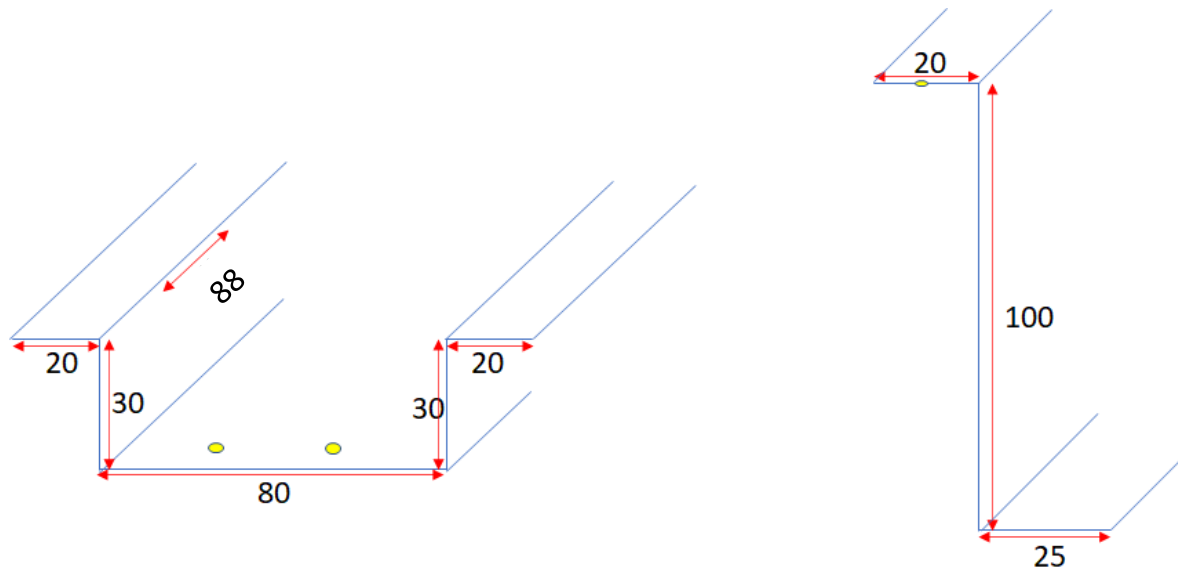


Figure S3: Dimensions of the aluminium logger frame. Left: the major part of the frame. The 80 mm part fits in the 90 mm part of a nest box and the 88 mm part in the 125 mm part. The 20 mm wings fit over the edges of a nest box. Right: side view from the back of the aluminium frame. Not shown: the length is slightly smaller than 80 mm, and fits in the 80 mm width of the left part. Figure and measurements were made by masters student Nivard Boersma.

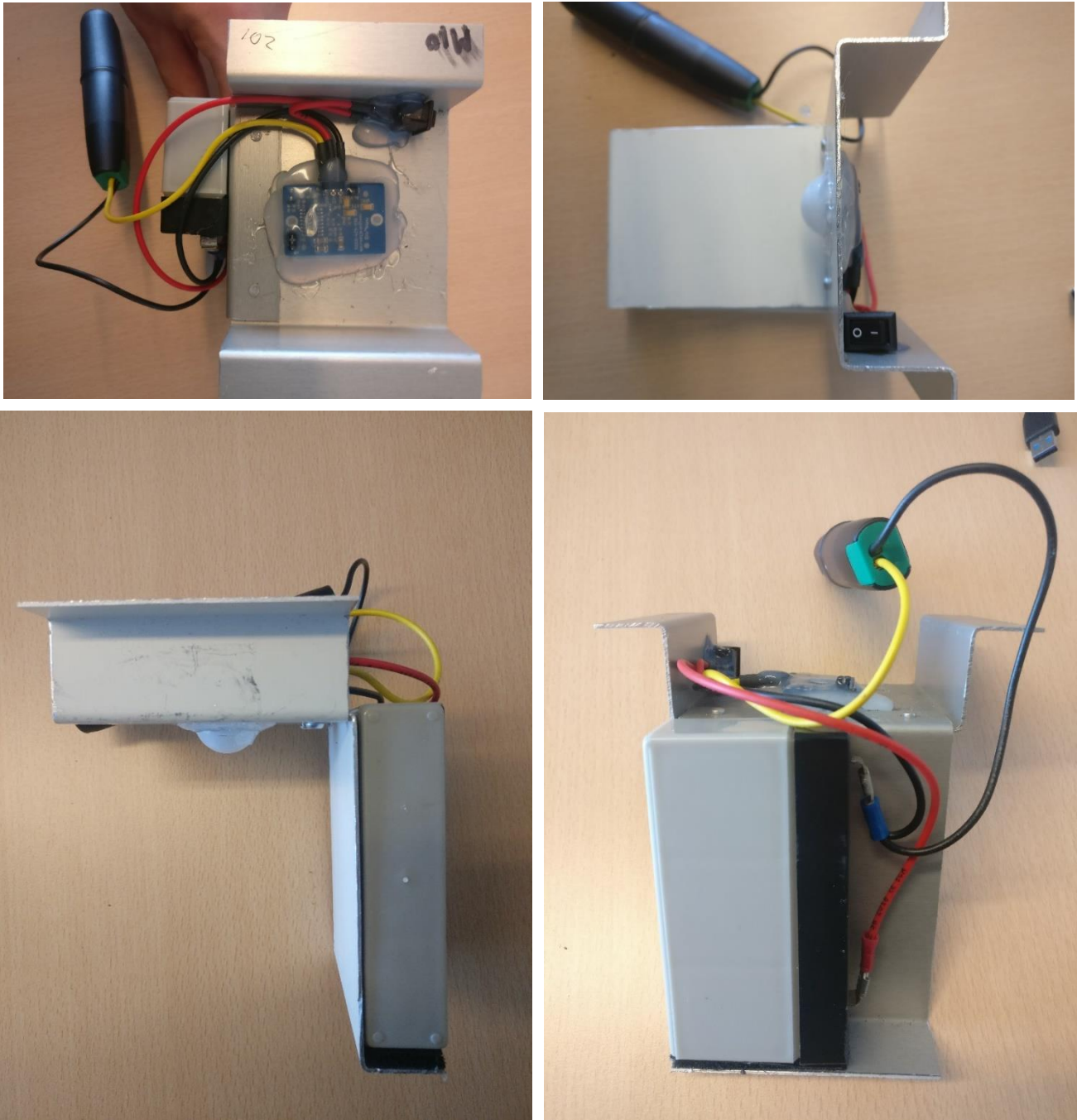


Figure S4: Logger design. To measure nest box occupancy and the timing of bird activity, we developed loggers that detect movement in a nest box. A logger consisted of a wide-angled infrared movement detector, a power source, and a data logger, attached to an aluminium frame. The movement detector and on/off switch are glued onto the aluminium frame. The layer of glue is protective against moisture. The power source is attached to the frame by a velcro strap. The movement detector, power source and data logger are connected by electric wires via soldering. Pictures were taken by masters student Nivard Boersma.

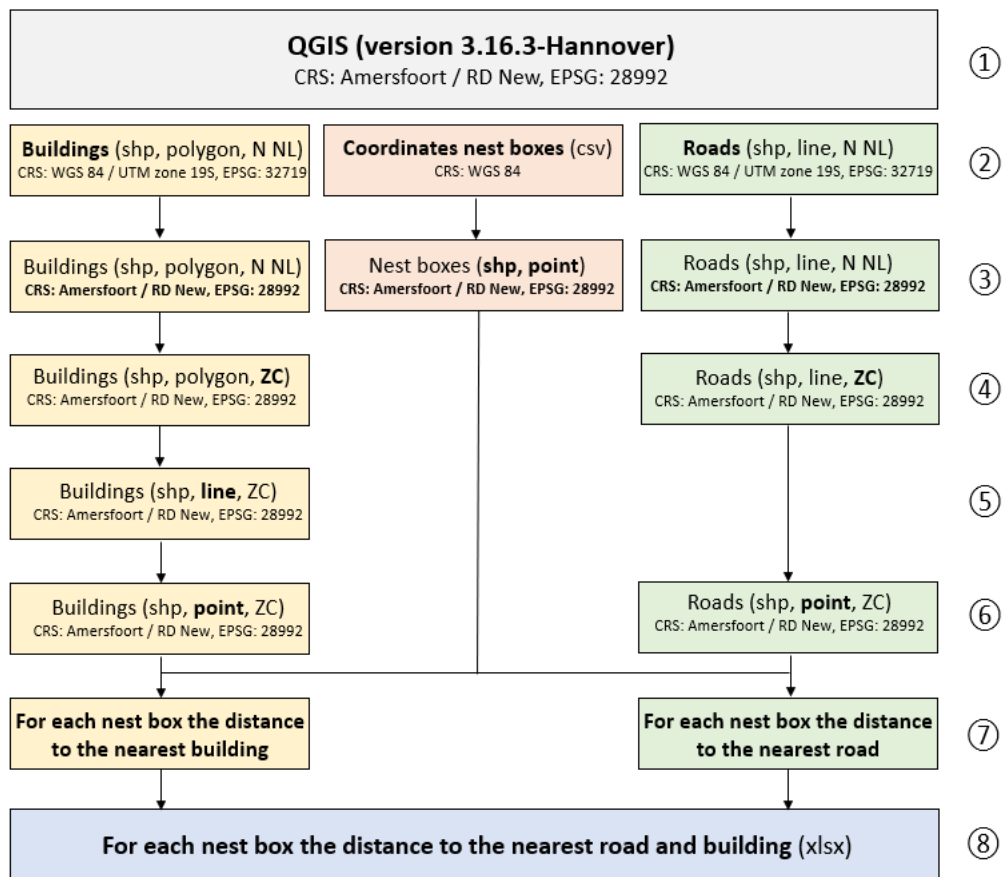
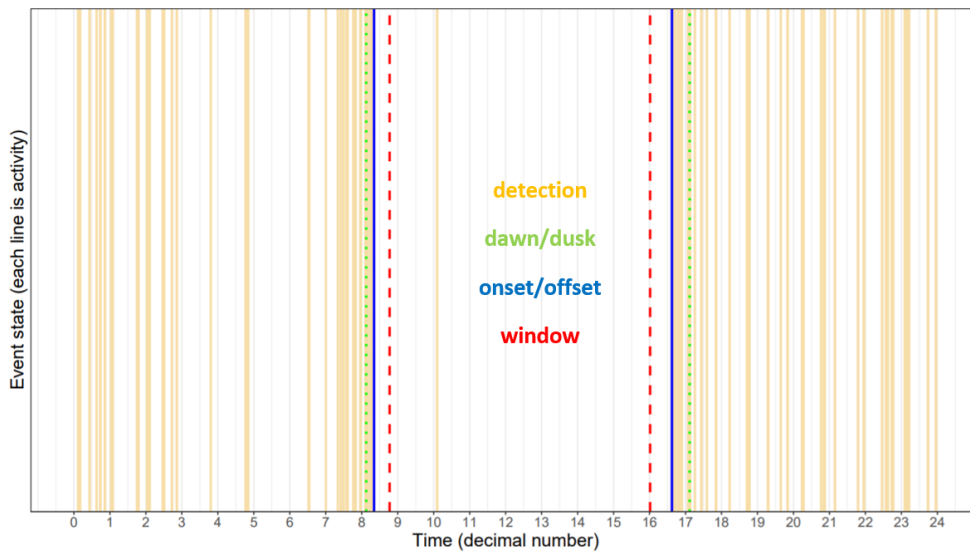


Figure S5: Flowchart of the calculation of the distances from the nest boxes to the nearest buildings and roads. Changes for each step are indicated in bold. ① The project was built in QGIS version 3.16.3-Hannover. The reference coordinate system (CRS) and datum were set to Amersfoort / RD New, 28992, which is the recommended format for the Netherlands. ② Building and road shapefile (shp) layers were added to the project as vector data layers. The data was accessed via download.geofabrik.de, and consists of polygons and lines, respectively. A csv file with the coordinates of the nest boxes was also added. ③ The CRS and datum of the layers did not correspond to those of the project. Therefore, the layers were transformed to the CRS and datum of the project (CRS: Amersfoort / RD New, EPSG: 28992). The nest boxes were in this step also saved as a shapefile consisting of points. ④ The building and road data layers contain all data from the northern Netherlands (N NL), but there was only data needed for the Zernike Campus (ZC). Therefore, a polygon was drawn in the size of the campus and the building and road data within the Zernike Campus polygon was selected by the “Select by Location” tool. Not shown: the Feringa Building was already part of the building layer, however, the construction of this building was not finished yet. Therefore, this polygon was removed from the dataset. ⑤ To calculate the distances from the nest boxes to the nearest buildings and roads, building polygons were first converted to lines with the “Polygons to Lines” tool. ⑥ Lines of the buildings and roads were converted to points with the “Lines to points” tool. The distance between the points was set to 1 m. ⑦ For each nest box, the distance to the nearest building and road was calculated with the “Distance to Nearest Hub (Points)” tool. ⑧ The distances were saved and combined in an Excel file.

Box 1: Selecting the best windows for the logger data.

A script was written in R to calculate the onset and offset of daily activity from the logger data. The morning window was set to 0.65 h after dawn, and the evening window to 1.10 h before dusk. In this box is explained how these windows were selected. The first part is an extended version from the text.

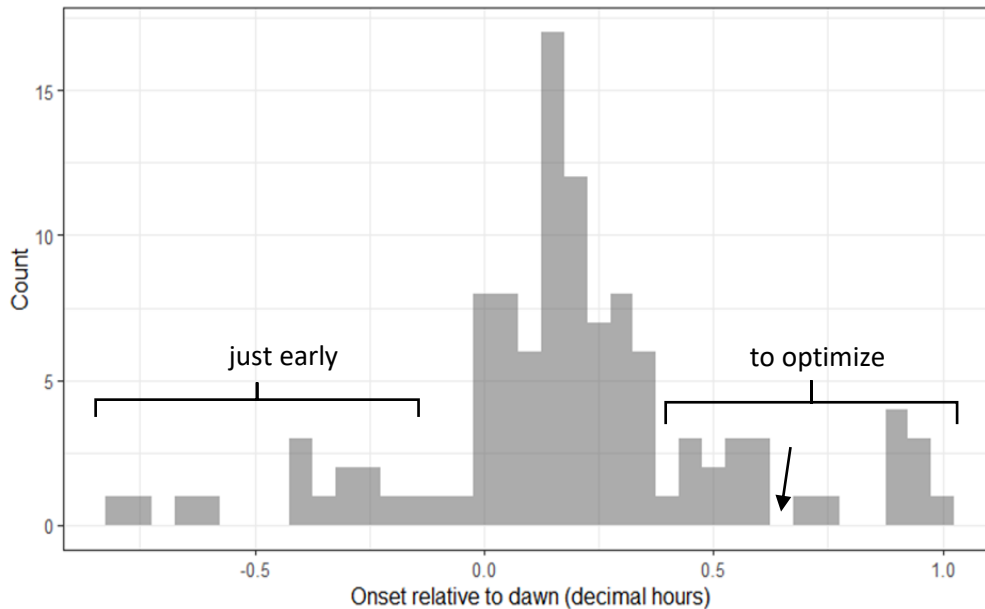
The onset of daily activity from the logger data is considered as the latest timepoint in a window after dawn, so when the bird left the nest box. The offset of activity is seen as the first timepoint in a window before dusk, so when the bird entered the nest box. As illustrated in the image of an output plot below, the onset (left blue line) is typically characterized as the datapoint at the end of a burst of detections (apricot lines) in the morning. Similarly, the offset (right blue line) is typically characterized by the first datapoint of many detections in the evening. In an ideal situation, there are not many detections after the onset and before the onset.



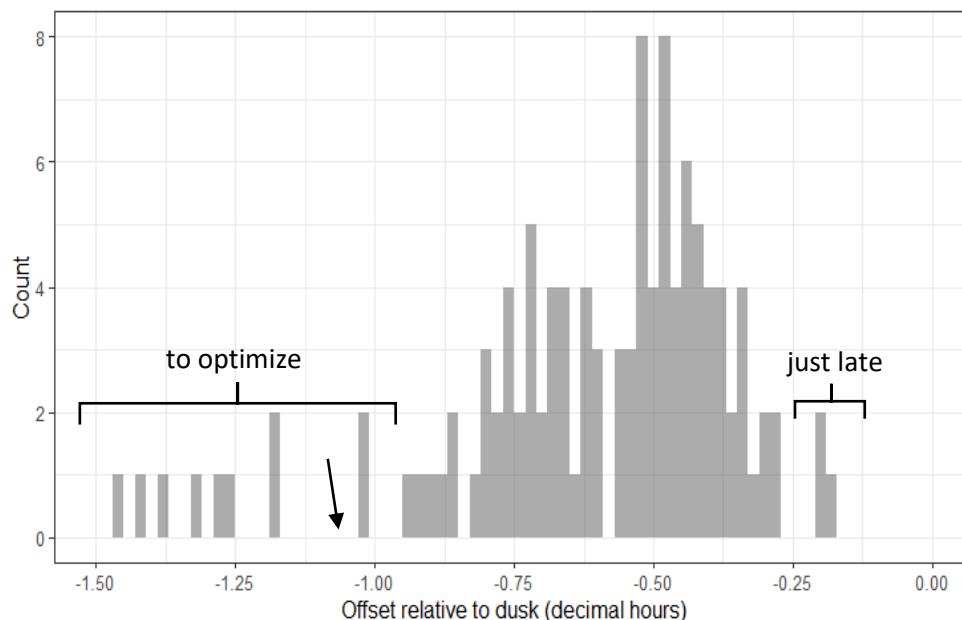
It often happens that a bird returns in a nest box after the onset, or comes by before the typical offset. When the window (red dashed lines) after a selected time after dawn/ before dusk (green dotted lines) is too large, these returns will be calculated at the onset/offset, as shown below on the left. However, when the window is too small, the real onset/offset is outside the window, as shown below on the right. The onset and offset that visual inspection suggest are indicated with an arrow.



While selecting the best window, one should aim to minimize the under- and overestimated onsets and offsets. Window optimization was based on distributions of the onset and offset (in hours to dawn/dusk) with a too large window (here 1 h after dawn and 1.5 h before dusk), as shown below. The left tail of the onset histogram contains just early birds, which are not affected by large window. However, when the window is too large, one ends up with a right tail of late onsets. This right tail is where the window should be optimized.



The right tail of the offset histogram contains late birds, for which we do not have worry. However, the left tail contains offsets that are too early, due to birds just coming by. Therefore, the left tail of the offset window needs optimization.



The histograms give an indication, but no definitive answer to the question when the windows should start and stop. Therefore, multiple possibilities were tried and the quality of the onsets and offsets were scored based on the output plots. This resulted in the windows of 0.65 h after dawn and 1.10 h after dusk, as indicated with arrows in the histograms.

Table S1: Overview of the division of sets and boxes into bird numbers. The boxes were assumed to be the same bird when there was an occupied night max 3 days later. Later than 3 days, it was assumed and treated as a different bird. This resulted in 15 birds.

Set	Box	Bird
1	115	1
1	194	2
2	106	3
2	115	1
2	123	4
2	133	5
2	194	6
4	151	7, 8
4	159	9
4	163	10
5	115	11, 12
5	160	13
5	162	14
5	164	15
6	115	12

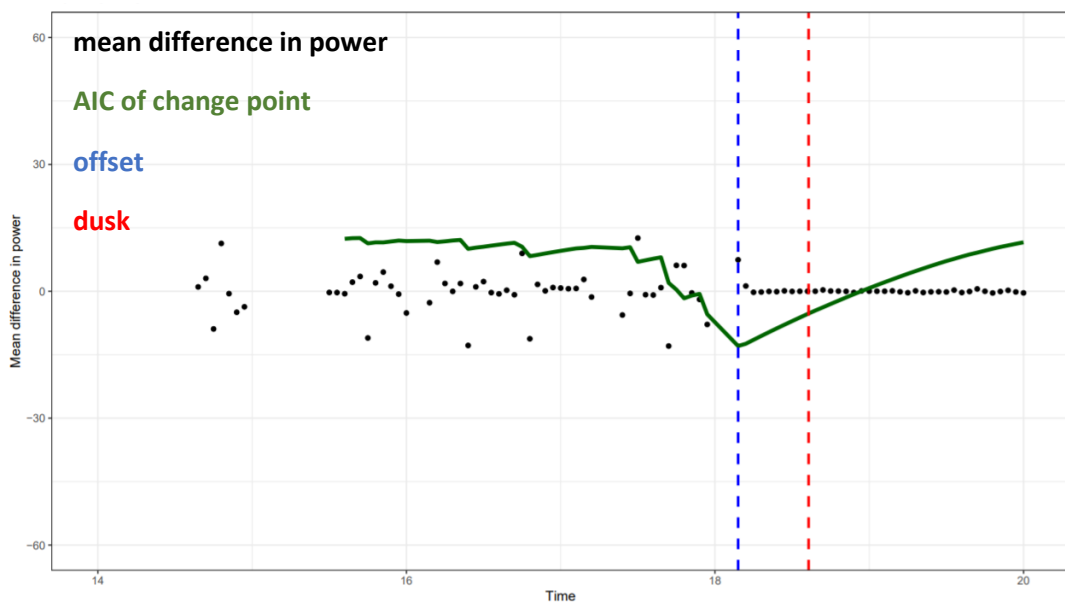
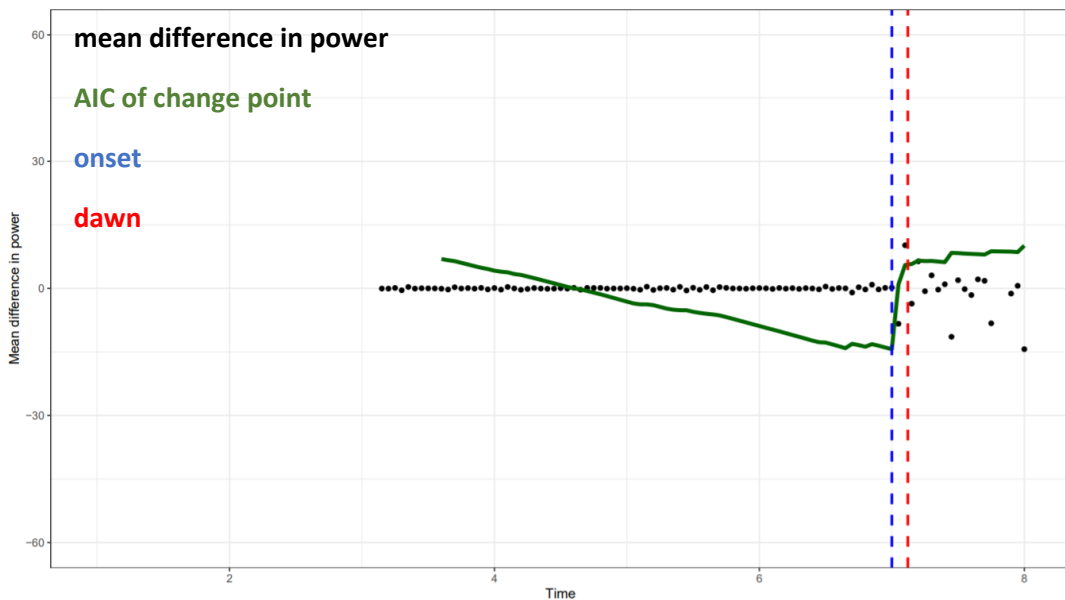
Table S2: Overview of the number of onsets, offsets and durations of daily activity from the logger data.

Bird	# Onset	# Offset	# Duration
1	22	23	20
2	2	2	1
3	10	9	8
4	2	2	1
5	10	10	9
6	9	10	8
7	2	2	0
8	1	1	0
9	12	12	11
10	2	2	0
11	2	2	0
12	25	25	23
13	1	1	0
14	10	10	9
15	2	2	0

Box 2: Visual correction of the onset and offset from the telemetry data

The onset and offset from the telemetry data were sometimes different than suggested by the output plots. In this box is explained how these data were corrected. The first part was already mentioned in the text.

The onset and offset of daily activity from the telemetry data were modelled in a behavioural change point analysis (BCPA). The BCPA assumed that the change in signal strength across time was drawn from two different normal distributions (one for the active and one for the non-active state) within a temporal window of 4 h before and 2 h after dawn/dusk. An Akaike information criterion (AIC) value was given to all possible change points in activity status. The timepoint that produced the lowest AIC value was identified as the onset/offset of activity. This is visualized in the output plots below (first onset, then offset). The mean difference in power over time (activity status) is indicated with the black dots. When the dots are flat the bird is considered as inactive, and when the dots are jumpy the bird is considered as active. The green line indicates the AIC value. The red dashed line indicates dawn/dusk and the blue dashed line the onset/offset (lowest AIC).



The model was not optimized for the calculation of the onset and offset of activity. Therefore, the model results sometimes needed correction by visual inspection of the output plots. The plot below is an example for when the modelled onset did not correspond with what is suggested by the plot. Namely, the modelled onset is around 06:15 h, whereas the change in activity status suggest that the onset is at the time of dawn. In these cases, the timepoint of the lowest AIC value that matched the visually inspected change in activity status (the second lowest valley of the green line, see arrow) was selected as the onset/offset.

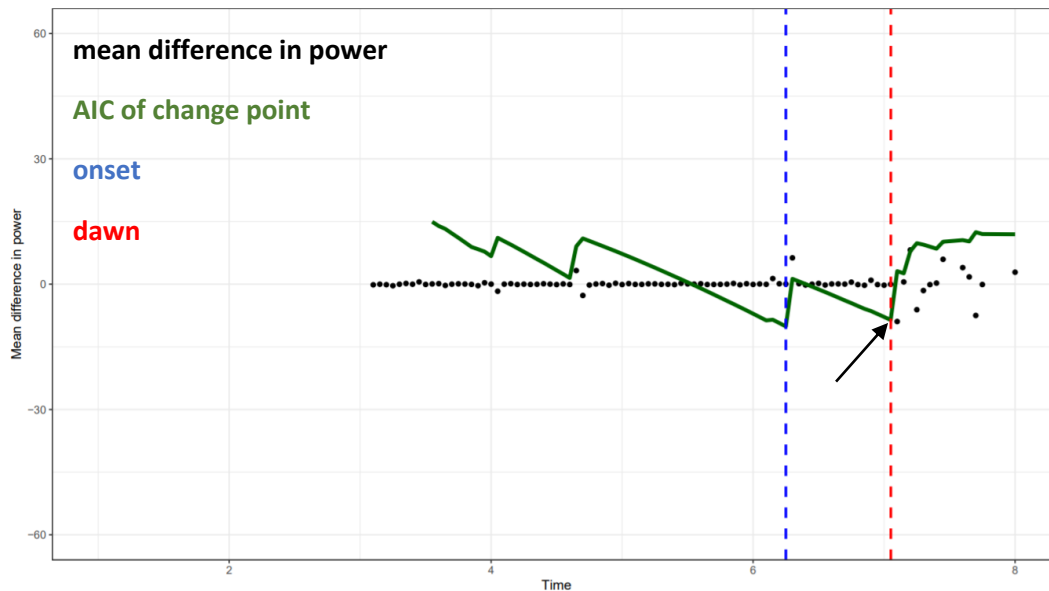


Table S3: Comparison telemetry and logger data. The telemetry data from 2021-02-24 (asterisk) seemed to be shifted one hour back in time (darker grey columns). Therefore, the data were corrected by adding one hour to the onsets and offsets. The raw data suggested that telemetry onset was earlier than logger onset, that the offset was variable, and that the duration was longer. "T" indicates telemetry and "L" indicates logger. The onset and offset are expressed in h:m:s time format. The duration is expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset).

Date	Onset T	Onset L	Offset T	Offset L	Duration T	Duration L	Onset T	Offset T
2021-02-20	07:00:00	07:11:18	NA	NA	NA	NA	07:00:00	NA
2021-02-21	06:54:00	07:12:33	17:57:00	18:04:54	96.43	94.88	06:54:00	17:57:00
2021-02-21	07:03:00	07:13:33	17:57:00	17:57:49	94.58	93.17	07:03:00	17:57:00
2021-02-23	06:51:00	07:07:24	18:09:00	18:09:58	97.49	95.27	06:51:00	18:09:00
2021-02-24*	07:09:00	07:19:07	18:09:00	18:04:25	94.36	92.26	06:09:00	17:09:00
2021-02-25	06:48:00	07:09:43	18:03:00	18:03:50	95.96	92.99	05:48:00	17:03:00
2021-02-26	06:54:00	07:07:20	18:09:00	17:59:18	95.42	92.16	05:54:00	17:09:00
2021-02-27	06:42:00	07:10:35	18:15:00	18:14:16	97.40	93.28	05:42:00	17:15:00
2021-02-28	06:45:00	07:05:50	18:09:00	18:04:39	95.60	92.07	05:45:00	17:09:00
2021-03-01	07:09:00	07:15:21	NA	NA	NA	NA	06:09:00	NA
2021-03-02	06:33:00	06:53:28	18:12:00	18:16:32	96.59	93.69	05:33:00	17:12:00

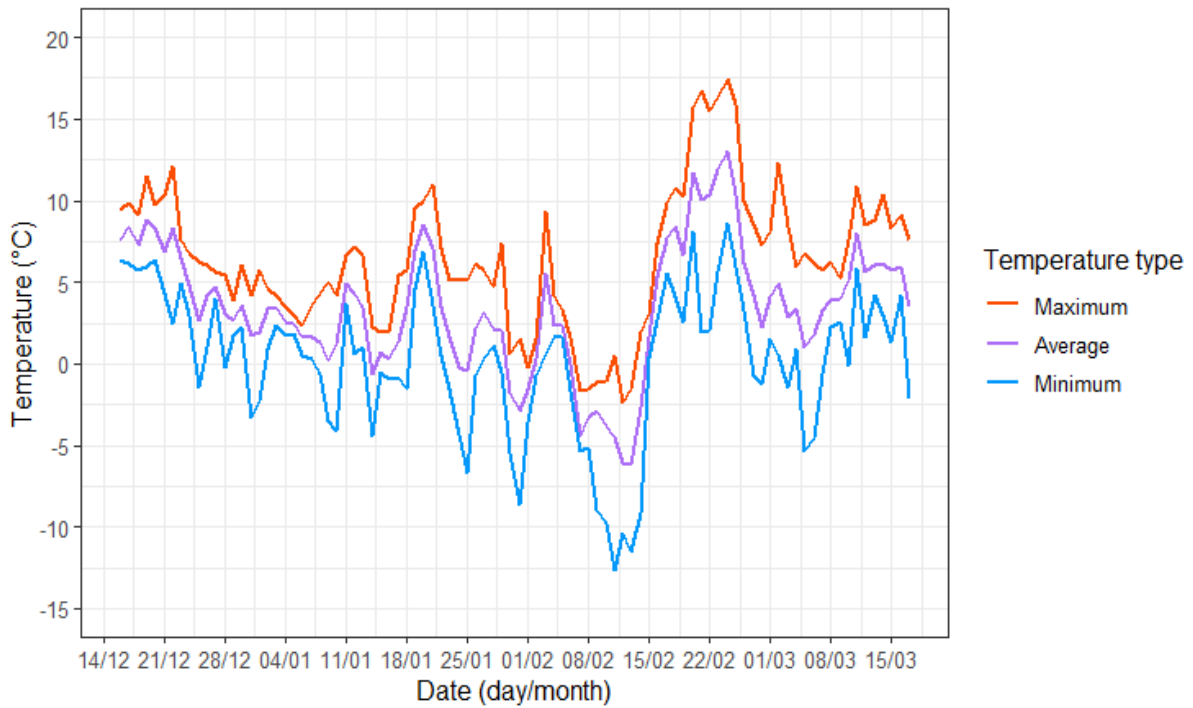
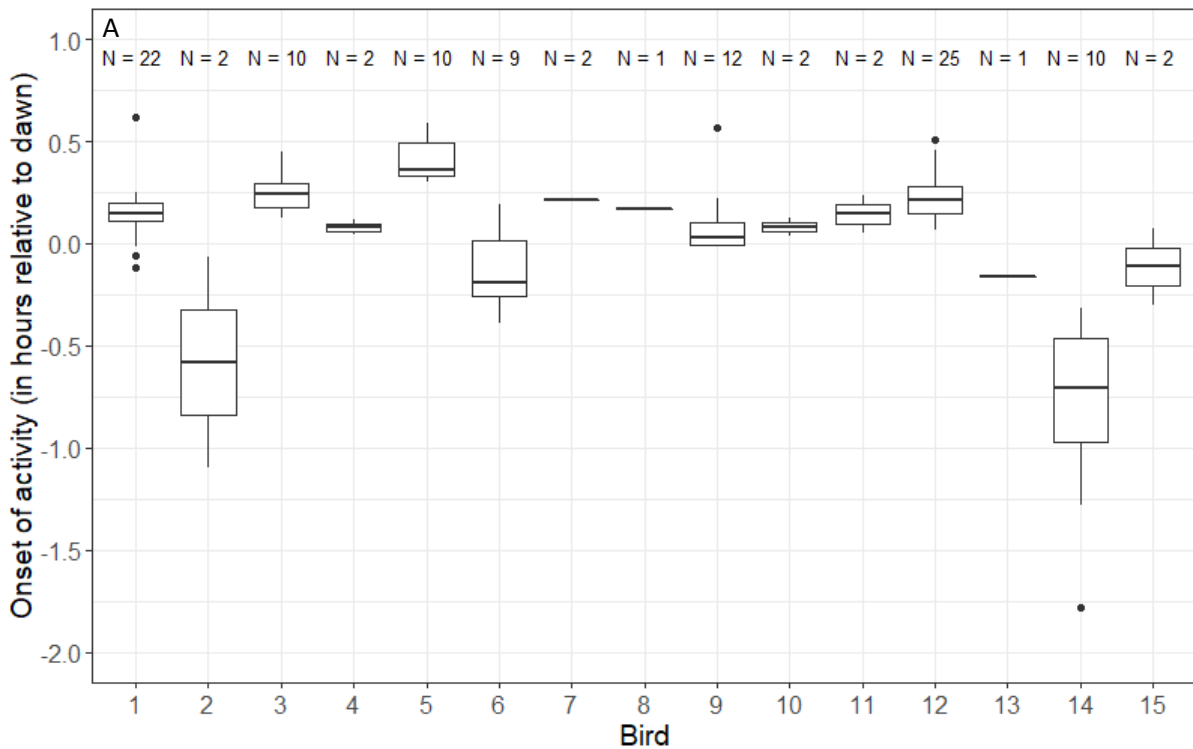


Figure S6: Average daily temperature over the course of the study period. In the week of 8-15 February we experienced a severe drop in temperature, which was followed up by an extreme rise in temperature. Temperature data (in degrees Celsius) was obtained from the Royal Dutch Meteorological Institute. The data was measured in Eelde, which is around 15 km south of the Zernike Campus. The red line indicates the maximum temperature, the purple one the average temperature, and the blue one the minimum temperature. The average temperature was used in the models.



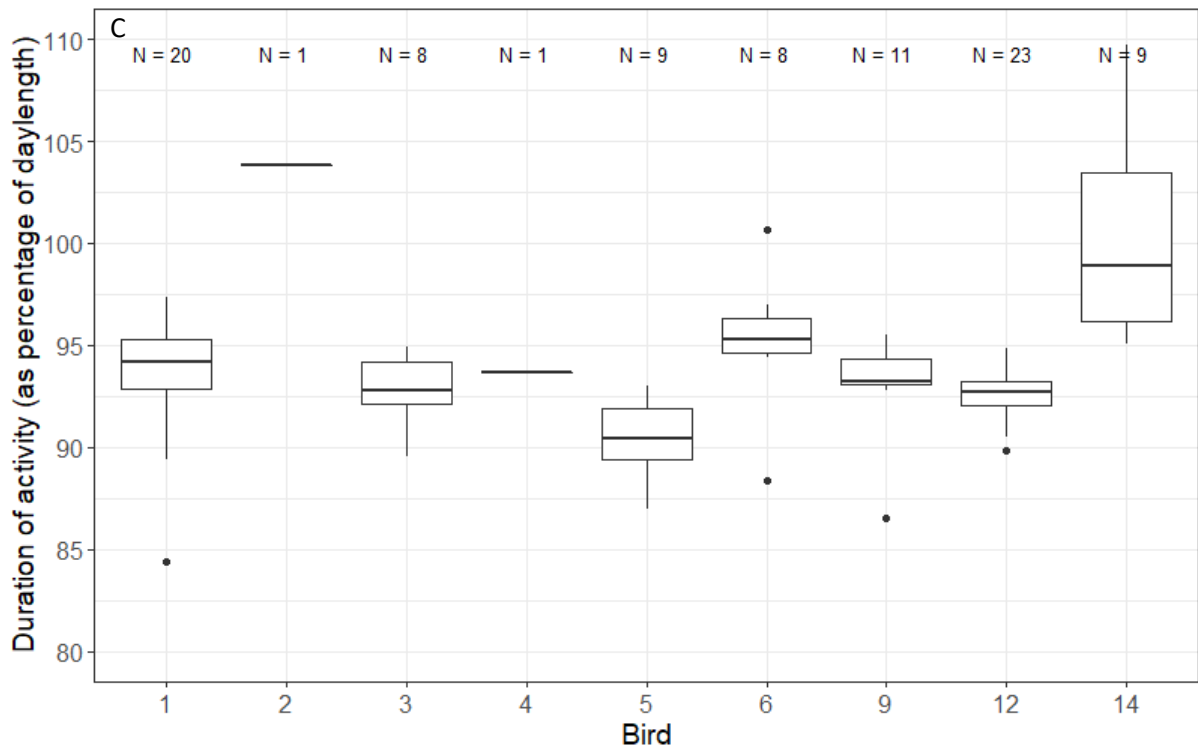
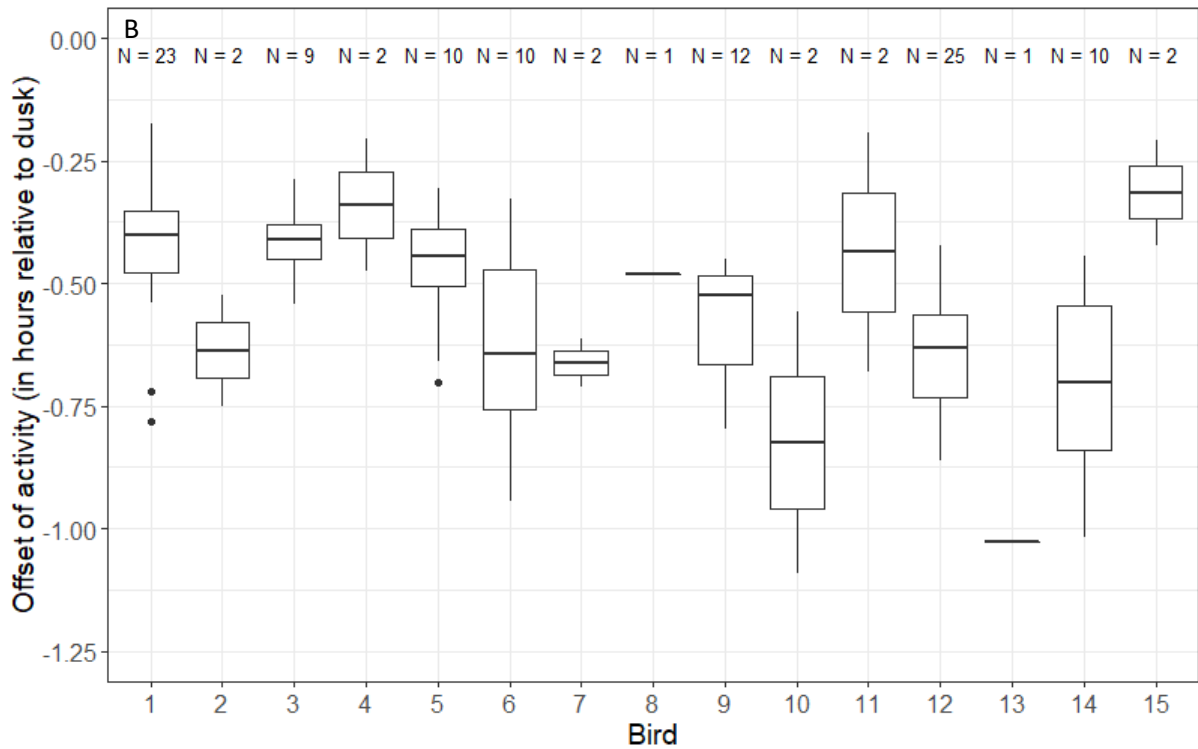


Figure S7: Within and between individual variation in activity measures. A) onset, B) offset, and C) duration. The onset and offset are expressed in hours relative to dawn and dusk, where a negative value means before dawn/dusk and a positive value after dawn/dusk. The duration is expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset). Note that figure C misses birds, because there were no sufficient onset and offset data to calculate the duration. The boxes indicate the 25th and 75th percentiles. Whiskers represent the highest and lowest values within 1.5 times the interquartile range from resp. the 3rd and 1st quartile. Dots are datapoints outside this range. The horizontal black lines within the boxes represent the median. The numbers above the boxes indicate the number of observations per bird.

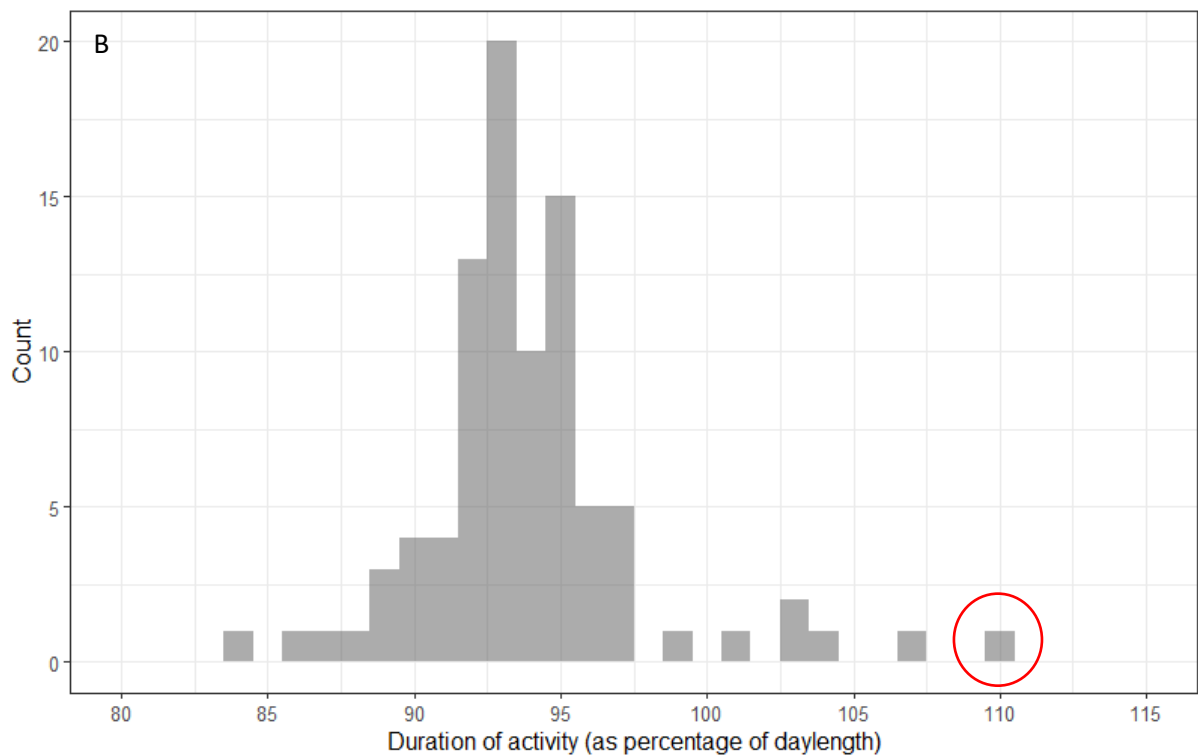
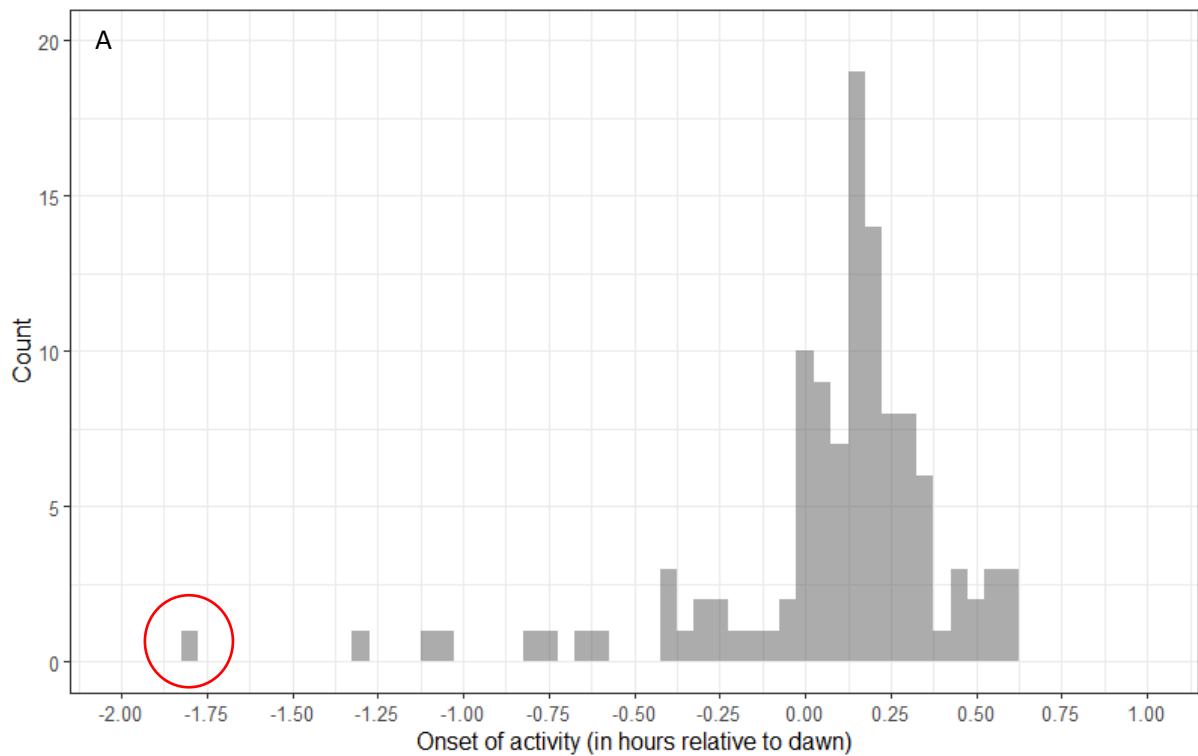
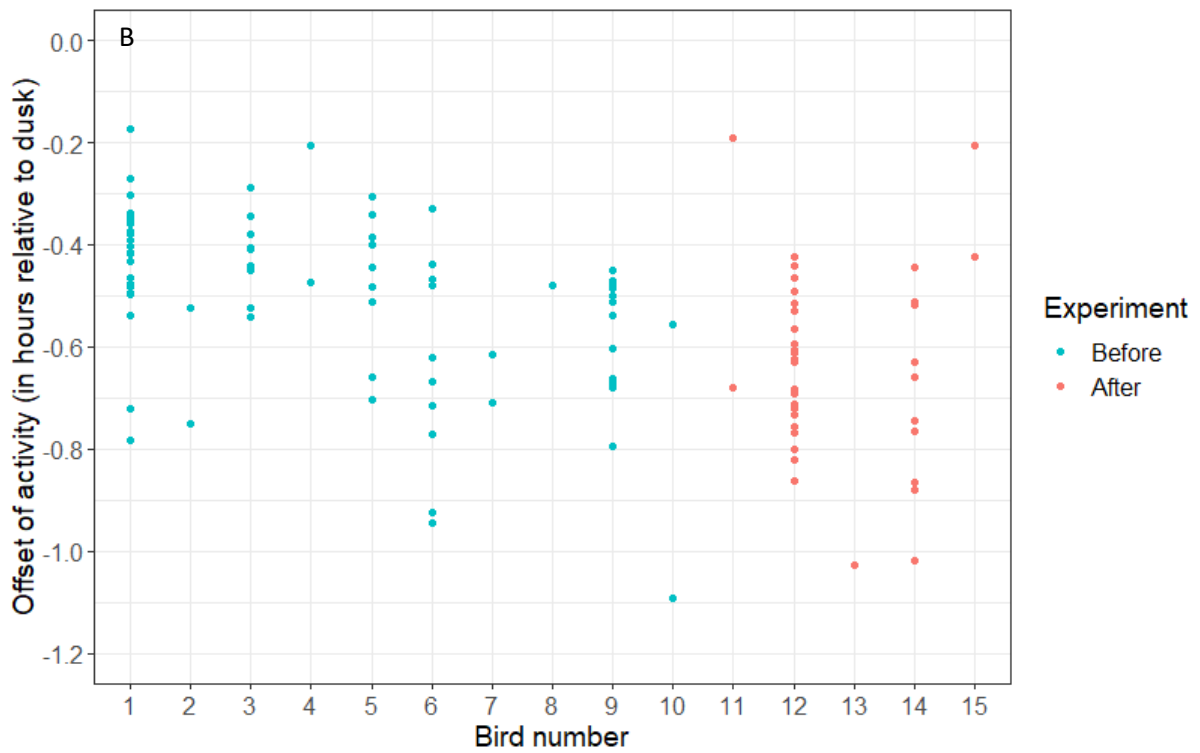
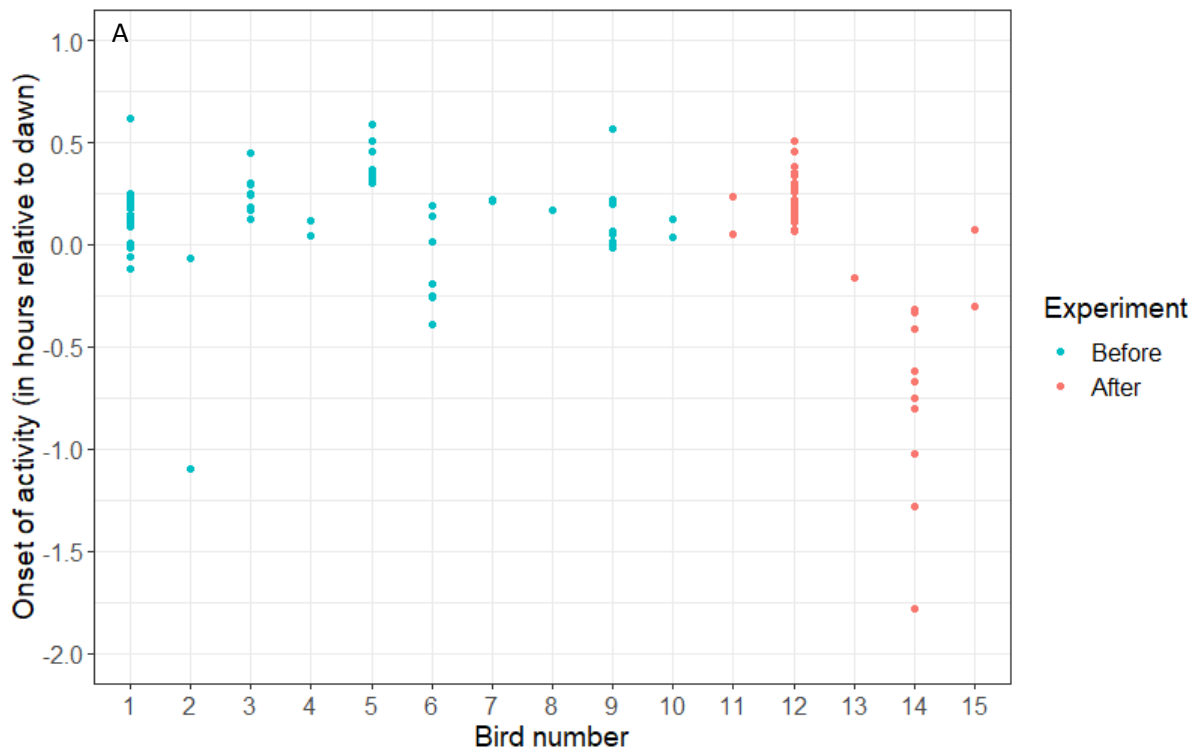


Figure S8: Outliers in the distributions of the onset and duration of activity. The models for the onset and duration of the active phase of the logger dataset did not meet the requirement of a normal distribution of the residuals. A) The left tail of the onset distribution contained one observation of a very early onset around 1.80 h before dawn. B) The right tail of the duration distribution contained one very long duration of activity, spanning 110% of the daylight fraction of the day. These outliers, indicated with a red circle around them, may have contributed to the deviation from a normal distribution in the models. They were therefore removed from the data in a subsequent analysis to test for the robustness of the initial model.

Table S4: The onset and duration of the active phase before and after the removal of one extreme outlier. The models for the onset and duration of the active phase did not meet the requirement of a normal distribution of the residuals. To test the robustness of these models, the most extreme left tail outlier was removed from the onset data (early bird), and the most extreme right tail observation was excluded from the duration data (long active period). The models were run again, and model parameters remained similar, indicating that the first, non-normal models were robust towards the outlier. Slope estimates are expressed in hours relative to dawn for the onset, to dusk for the offset, and percentages of the daylight fraction of the day for the duration. Slope estimates between parentheses indicate the non-log estimates in minutes (unless otherwise indicated). Models were additive linear mixed effects models, run with the lme4 package in R. Fixed effects were the light intensity (lux), the distance to the buildings (m) and the date (numeric, day 0 = 21 Dec 2020). Light intensity and distance to the nearest building were on natural logarithmic scales. Bird identity and average temperature were added as random effects. “r bird” and “r temp” indicate the repeatabilities (ICCs) for the bird number and the average temperature, respectively.

Model	Variable	Estimate	SE	df	t-value	p-value	2.5 – 97.5% CI	r bird	r temp
Onset	(Intercept)	0.56 (33.41)	0.44	14.09	1.25	0.23	-0.27 – 1.38	0.77	0.23
	Light intensity (log)	-0.060 (-3.57)	0.094	13.82	-0.64	0.54	-0.23 – 0.11		
	Dist. building (log)	-0.12 (-7.27)	0.13	12.80	-0.92	0.37	-0.36 – 0.12		
	Date	$4.25 \cdot 10^{-3}$ (0.26)	$2.99 \cdot 10^{-3}$	72.53	-1.42	0.16	$-9.94 \cdot 10^{-3}$ – $1.63 \cdot 10^{-3}$		
Onset without outlier	(Intercept)	0.51 (30.31)	0.41	13.96	1.24	0.24	-0.24 – 1.26	0.80	0.29
	Light intensity (log)	-0.053 (-3.16)	0.086	13.64	-0.61	0.55	-0.21 – 0.11		
	Dist. building (log)	-0.12 (-7.03)	0.12	12.65	-0.97	0.35	-0.34 – 0.10		
	Date	$2.97 \cdot 10^{-3}$ (-0.17)	$2.65 \cdot 10^{-3}$	77.22	-1.12	0.27	$8.01 \cdot 10^{-3}$ – $2.15 \cdot 10^{-3}$		
Duration	(Intercept)	84.48 (8.67 h)	8.33	5.97	10.13	$5.55 \cdot 10^{-5}$	70.13 – 98.90	0.78	0.38
	Light intensity (log)	0.72 (4.42)	1.94	5.72	0.37	0.73	-2.63 – 4.08		
	Dist. building (log)	2.65 (16.32)	2.49	5.86	1.06	0.33	-1.65 – 6.94		
	Date	0.075 (0.46)	0.043	37.96	1.74	0.090	-0.011 – 0.15		
Duration without outlier	(Intercept)	85.77 (8.80 h)	7.62	5.95	11.25	$3.11 \cdot 10^{-5}$	72.64 – 98.93	0.80	0.46
	Light intensity (log)	0.47 (2.88)	1.77	5.69	0.26	0.80	-2.59 – 3.54		
	Dist. building (log)	2.39 (14.71)	2.27	5.82	1.05	0.34	-1.53 – 6.31		
	Date	0.056 (0.34)	0.040	36.69	1.41	0.17	-0.020 – 6.31		



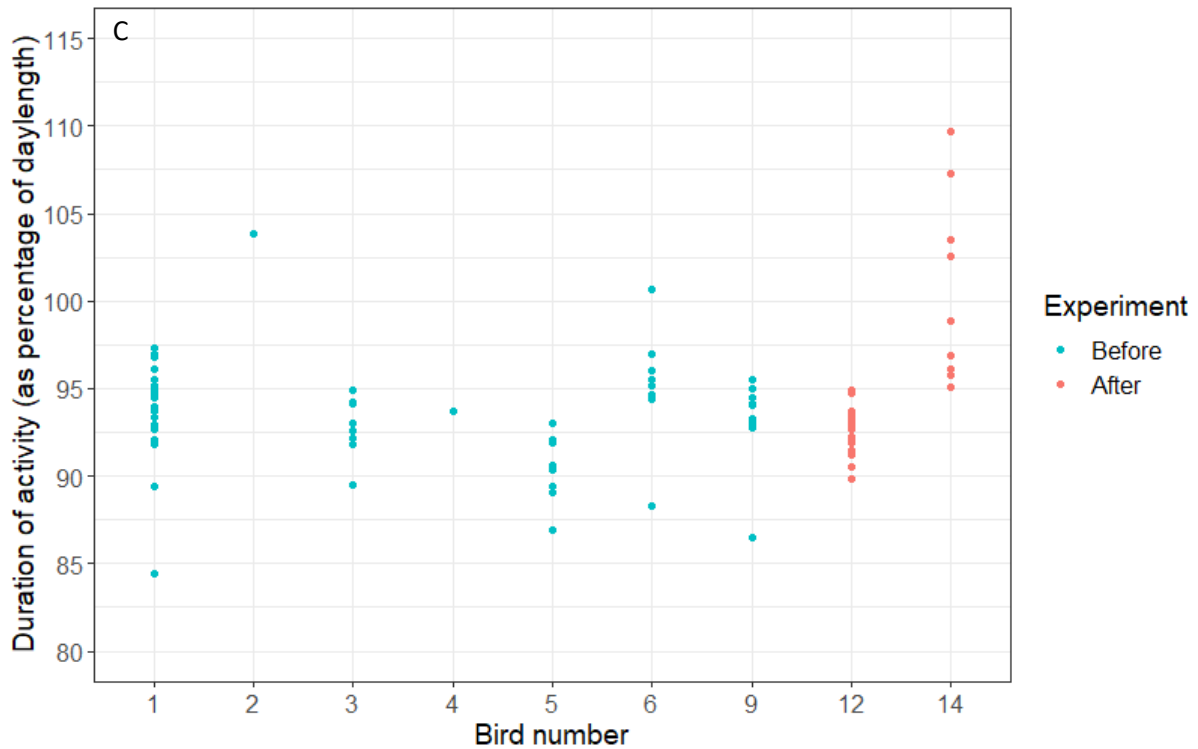


Figure S9: Activity timing before and after the dim-light experiment. A) onset, B) offset, and C) duration. 13 Birds were present in dim-light from 19 January to 4 February to measure their internal rhythm in dim-light. This might have influenced the timing of the onset and offset, and the duration, because birds returning from constant dim-light conditions may have had different rhythms than birds that were continuously exposed to external cues at the campus. We did not know whether the birds that returned from the experiment were present in our nest boxes (except for bird 12), so we visually compared the activity measurements of all birds before the experiment (blue dots) with all birds after the experiment (red dots), using the logger dataset. Eyeballing the plots suggested that the overall timing of activity remained similar. Bird 14 was early in the morning, and had a long duration of the active phase. Imagining that this bird has been in the dim-light conditions, its measured activity was probably not affected by the experiment anymore, because the first activity measurement of this bird was 9 days after the experimental birds were released (not shown). So, it is very likely that bird 14 was just an early bird. The onset and offset are expressed in hours relative to dawn and dusk, where a negative value means before dawn/dusk and a positive value after dawn/dusk. The duration is expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset). Note that figure C misses birds, because there was not sufficient onset and offset data to calculate the duration.

Table S5: Logger and telemetry comparisons of activity timing. All slopes are smaller than 1. The average daily temperature (Avg T) does not explain much of the variation between the logger and telemetry data. The onset and offset are expressed in hours relative to dawn and dusk, where a negative value means before dawn/dusk and a positive value after dawn/dusk. The duration is expressed as the percentage of the daylight fraction of the day (dusk-dawn) the birds were active (offset-onset). These models were run as linear models in R, and the average daily temperature was added as an interaction term. The models contained the telemetry data with the 1 h correction in time. Rows with Avg T contain the main effect of the average temperature, and the Interaction rows the interaction between the telemetry data and the temperature.

Model	Variable	Estimate	SE	t-value	Pr(> t)	2.5 – 97.5% CI	F-stat	df	R ²	p-value
Onset	(Intercept)	0.25	0.024	10.30	2.79*10 ⁻⁶	0.19 – 0.30	14.75	1, 9	0.62	3.96*10 ⁻³
	Telemetry	0.53	0.13	3.84	3.96*10 ⁻³	0.22 – 0.84				
Onset temperature	(Intercept)	0.33	0.047	7.12	1.89*10 ⁻⁴	0.22 – 0.44	8.80	3, 7	0.79	8.97*10 ⁻³
	Telemetry	0.31	0.24	1.26	0.26	-0.27 – 0.88				
	Avg T	-0.011	5.34*10 ⁻³	-1.99	0.087	-0.023 – 2.00*10 ⁻³				
	Interaction	0.030	0.031	0.94	0.38	-0.045 – 0.10				
Offset	(Intercept)	-0.15	0.25	-0.60	0.57	-0.73 – 0.43	3.06	1, 7	0.30	0.12
	Telemetry	0.77	0.44	1.75	0.12	-0.27 – 1.80				
Offset temperature	(Intercept)	0.11	0.70	0.16	0.88	-1.67 – 1.90	1.47	3, 5	0.47	0.33
	Telemetry	1.36	1.22	1.12	0.32	-1.78 – 4.51				
	Avg T	-0.036	0.070	-0.51	0.63	-0.22 – 0.15				
	Interaction	-0.082	0.13	-0.65	0.55	-0.41 – 0.24				
Duration	(Intercept)	26.78	27.62	0.97	0.36	-38.54 – 92.09	5.80	1, 7	0.45	0.047
	Telemetry	0.69	0.29	2.41	0.047	0.013 – 1.37				
Duration temperature	(Intercept)	52.95	62.65	0.85	0.44	-108.09 – 214.00	6.05	3, 5	0.78	0.041
	Telemetry	0.41	0.65	0.62	0.56	-1.26 – 2.08				
	Avg T	-4.15	6.05	-0.69	0.52	-19.71 – 11.41				
	Interaction	0.045	0.063	0.71	0.51	-0.12 – 0.21				

Table S6: Logger and telemetry comparisons of uncorrected activity timing. The linear models without correction contained the telemetry data with the 1 h shift in time from 24 February onwards, which made the telemetry data much earlier than the logger data. As a result, the slope estimates became smaller and less realistic than the estimates in table S5. The onset is expressed in decimal hours relative to dawn, where a negative value means before dawn, and a positive value after dawn. The offset is similarly expressed relative to dusk.

Model	Variable	Estimate	SE	t-value	Pr(> t)	2.5 – 97.5% CI	F-stat	df	R ²	p-value
Onset uncorrected	(Intercept)	0.15	0.060	2.44	0.038	0.01 – 0.28	2.04	1, 9	0.18	0.19
	Telemetry	-0.10	0.073	-1.43	0.19	-0.27 – 0.060				
Offset uncorrected	(Intercept)	-0.43	0.072	5.95	5.71*10 ⁻⁴	-0.60 – -0.26	4.79	1, 7	0.41	0.065
	Telemetry	0.12	0.055	2.19	0.065	-9.97*10 ⁻³ – 0.25				

Acknowledgements

Throughout my minor research project, I have received much support and assistance.

I would first like to thank my supervisor, prof. dr. Barbara Helm, for her guidance through each stage of my project. Your expertise in the field, keen interest in my project, trust in my capabilities, and insightful feedback have helped me very much and brought my work to a higher level. I also really appreciate the chances you have given me outside this project. Dear Barbara, it was a pleasure to work with you.

I would also like to thank you, Aurelia Strauß (Barbara's PhD student, my co-supervisor), for all the work you have done to let me use your telemetry data, for teaching me how to locate the birds, for your advise during my project and feedback on my report. I have enjoyed learning radio telemetry and I wish you the best of luck with the continuation of your PhD.

Next, I would like to thank Nivard Boersma, a masters student in the group of Barbara. It was great that I could use your logger and light intensity data for my project, and that you taught me how to use the loggers for some additional data collection. Thereby, your company and assistance with learning telemetry and locating the birds have made the cold days and evenings much better.

I am really grateful that Pablo Capilla-Lasheras, Davide Dominoni, and Cara Cochrane from the University of Glasgow were willing to share their scripts with the preparations for and the running of the behavioural change point analysis with me. Help of the GeoDienst of the University of Groningen was also much appreciated and really valuable for getting access to the spatial data and solving problems with the calculation of distances. I could not have done this all by myself.

Last but not least, I would like to thank my parents, my sister, and good friend Dana for their support. You were always there for me when I needed to talk about the achievements I made, but also about the issues I faced. You encouraged me to keep believing in myself when I was stressed, and I am really happy with all the nice distractions you gave me to clear my mind.