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DIEL ACTIVITY RHYTHMS IN WILD SONGBIRDS IN THE SUBARCTIC

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Abstract

The highly rhythmic environment caused by the rotation of the earth has driven the evolution of an intrinsic timekeeping mechanism in most organisms known as circadian clocks. As climate change drives the expansion of southern species into northern latitudes, their circadian clocks are challenged by the novel light conditions they encounter. In contrast to the clear day and night in southern latitudes, the light-dark regime of the Arctic environment is characterised by a reduced daily amplitude of solar illumination, including periods where the sun remains continuously below (polar night) and above (polar day) the horizon, and rapid changes in daylength in between. This may lead to disruption of the circadian organisation with consequence of correct timing of behaviour. Here we observed the diel activity rhythms of three wild songbird species, the great tit, blue tit, and willow tit, which have colonised northern latitudes at different times. Daily activity was obtained through foraging activity using camera traps. We observed a distinct difference in diel activity rhythms between the newer coloniser, great and blue tit, from the well-established species, willow tit, of northern areas during polar day and polar night. We observed an activity pattern similar to a relative coordination for the great and blue tit population during polar night, which may suggest times of circadian disruption. In contrast, willow tit maintains robust entrainment throughout the year. Overall, our observation may indicate distinct differences in circadian clock properties between the populations, which may related to the time they have colonised the northern latitudes. Willow tits ability to maintain entrained when the daily amplitude of solar illumination is reduced suggest that the circadian system is more sensitive to light cues as an adaption to the light regime in the Arctic.

Keywords: Songbirds, diel activity rhythms, subarctic, natural light-dark cycle, entrainment, circadian

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Glossary

Circadian rhythm	An endogenous, self-sustained rhythm with a period of approximately 24-hour that can be entrained by a Zeitgeber.
Diel	Having a duration of a 24-hour day.
Entrainment	The synchronisation of the self-sustained rhythm (e.g. circadian) to environmental cycle (Zeitgeber).
Free-running	The state of self-sustained rhythm in the absence of effective Zeitgebers or other environmental cues that may affect the period.
Oscillator	An entity capable of generating spontaneous rhythms (e.g. circadian clock).
Photoperiod	Portion of daylight during a 24-hour day.
Relative coordination	The zeitgeber is too weak to entrain the self-sustained rhythm, however it still influence the phase of the rhythm.
Zeitgeber	External or environmental factor that synchronise a self-selfsustained rhythm

1 Introduction

1.1 Environmental cycles

The Earth's continuous rotation around its own axis and its orbital journey around the sun lead to cycles in environmental conditions such as light, temperature, and humidity. Consequently, these environmental cycles lead to fluctuations in resources, such as food quality and quantity. The planet completes a full rotation in approximately 24 hours, resulting in distinct variations in light throughout the day and creating the familiar cycle of day and night. Simultaneously, the earth uses a year to orbit the sun, which gives rise to seasons with varying daylength (photoperiod). The earth is tilted approximately 23.5° relative to the sun, consequently, part of the planet has less light when the tilt is away from the sun (winter solstice) and the opposite when it's tilted towards the sun (summer solstice) (Fig. 1). This gives rise to seasonal changes in photoperiod, which increase with latitude away from the equator (0°) towards the poles. At the equinoxes, the day and night are roughly the same in duration across all latitudes. However, latitudes above the Arctic Circle (66.33 °N) and below the Antarctic Circle (66.33 °S) undergo pronounced changes in photoperiod. The traditional distinction between "day" and "night" becomes blurred during parts of the year when the sun never rises above the horizon (polar night) and also when it never sets (polar day).

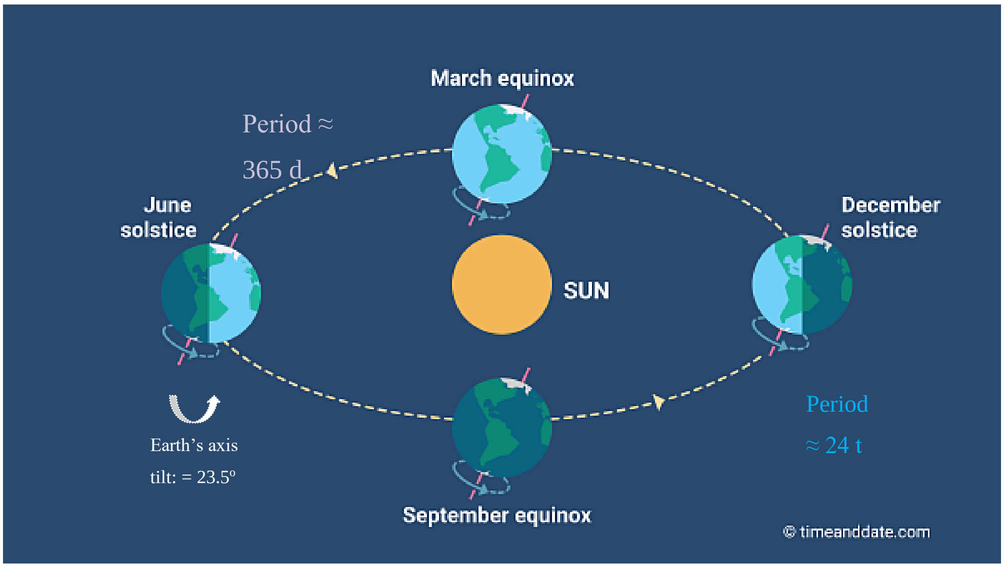


Figure 1: Earth´s orbit around the sun and its rotation around its own tilted axis. The earth completes an orbit around the sun in approximately 365 days and rotates around its own tilted axis approximately once every 24 hours, creating seasons but also day and night. Figure slightly modified from timeanddate.com.

1.1.1 Arctic environment

There are different definitions of "Arctic". Geographically, the Arctic is the area north of the Arctic Circle, which is located above 66.33° north from the equator. Another common definition in ecology is the 10 °C isotherm, which means that the average temperature do not exceed the 10 °C mark in July. Furthermore, based on climate and vegetation, the Arctic can be divided into three zones: high Arctic, low Arctic, and Subarctic (Blix, 2005). However, in my thesis, I will use the geographical definition of the Arctic, whereby regions located above the Arctic Circle experience annual fluctuations in photoperiods, including polar day and polar night, as previously mentioned.

The Arctic environment is anything but static, with daily fluctuations in insolation, solar elevation, and temperature occurring throughout both polar nights and polar days. Sunlight illumination in the day, depending on if it is cloudy or a clear day, ranges from 1000 to 100 000 lux (Longcore and Rich, 2016). The amount of sunlight illumination depends on the sun elevation, where the sun elevation below the horizon gives rise to three distinct twilight phases: civil twilight, nautical twilight and astronomical twilight. With a natural light-dark cycle, the light intensity changes most rapidly during civil twilight (Daan & Aschoff, 1975).

The duration of polar night and polar day, and duration of twilights expands as one moves towards higher latitudes, and the closer you get to the poles, the more pronounced the changes in daylength become (Fig. 2). Additionally, the extent of darkness experienced during polar nights depends on latitude. Areas closer to the Arctic Circle do not descend into total darkness because they still encounter civil twilight during midday and will be referred as polar twilight (Fig. 2C). The sun position also gives rise to daily changes in both spectral composition and light intensity during polar day.

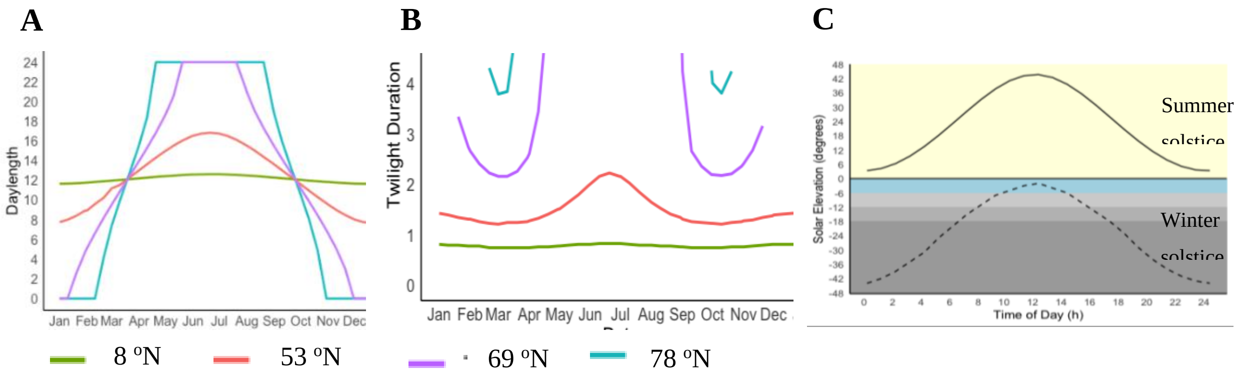


Figure 2: Annual variation in photoperiod and twilight duration at different latitudes, and sun elevation at summer and winter solstice. A. Photoperiod from sunrise to sunset B. twilight duration represents the time between nautical twilight (-12°) and sunrise (0°) **C.** Sun elevation at summer and winter solstice at 69 ° N, and different phases of twilight. Sun is above the horizon (yellow), civil twilight: 0 < θ < -6 (light blue), nautical twilight: -6 < θ <-12 (light grey), astronomical twilight: -12 < θ < -18 (grey), and night phase: - 18 < θ (dark grey).

1.2 The inner timekeeping system

The highly rhythmic environmental cycles caused by the earth's movement makes it challenging for animals to time their daily and annual events at the right time, and thereby maximize opportunities and chances of survival. For example, correct daily timing is important to reduce both predation risk and competition and, conversely, maximize foraging. Timing life history events with environmental conditions is crucial. For instance, timing reproduction or migration with the peak of food availability (quality and abundance), is a key factor to successful offspring (Siikamaki, 1998; Both & Visser, 2005).

Organisms have evolved an inner timekeeping system to adjust their behaviour and physiology to the day-night cycle. This system allows them to anticipate environmental changes for timing life events like moulting, migration, hibernation, and reproduction as well as daily timing in behaviour and physiology, e.g. foraging and sleep-wake cycle (Moore-Ede et al., 1982). Optimal timing of such daily events can significantly minimize predation risks (DeCoursey et al., 2000). The inner timekeeping system comprises three elements: 1) a sensory system that receives input from the environment, 2) an endogenous, self-sustained oscillator, and 3) output of physiological and behavioural rhythms (see Figure 3). The sensory system receives environmental cues, primarily from the light-dark cycle, acting as synchronising signals known as *Zeitgebers* (the German word for "time giver"). An endogenous oscillator (also often referred to as the innate clock) maintains an intrinsic rhythm in constant conditions but can be entrained by *Zeitgebers* to synchronise with the environment. Output pathways link the innate clock to physiological processes and behaviour, resulting in cyclic changes in behaviour and physiology, known as biological rhythms. These rhythms can have different periods, such as ultradian, circadian, tidal, and circannual.

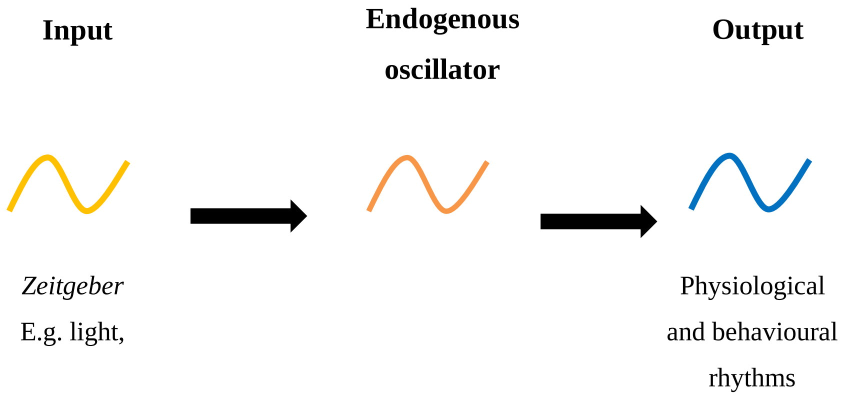


Figure 3: Eskinogram of the three components of the inner timekeeping system. Arrow represents pathways connecting the inner timekeeping system together.

The endogenous oscillator and rhythms relating to daily timing is often referred to as circadian clock and circadian rhythms. It is important to note that for a rhythm to be considered circadian, it must fulfil three criteria. 1) The circadian clock can be entrained by *Zeitgebers* to synchronise its phase with the environment, and 2) the rhythm should persist in the absence of *Zeitgebers* with a period of approximately 24 hours (free-running period). 3) The last criterion is temperature compensation, meaning it maintains a consistent period regardless of fluctuations in ambient temperature (Moore-Ede et al., 1982). The term "circadian" is commonly used, however it should only describe an organism that maintains an approximately 24h-rhythmicity under constant conditions. If there is no evidence of a self-sustained rhythm in constant condition, it cannot be known if the observed rhythmicity is driven by an endogenous oscillator or if it is an exogenous rhythm in direct response to the environment (*i.e.* masking). In this case, terms such as diel or daily should be used instead.

1.2.1 The circadian clock

The circadian clock is a molecular clock based on transcriptional-translational negative feedback loops (Buhr & Takahashi, 2013). In mammals, this system is highly centralized, with the suprachiasmatic nucleus (SCN) serving as the master clock (Dibner et al., 2010). In contrast, the avian circadian system consists of three major components: the pineal gland, the hypothalamic region (analogous to the SCN in mammals), and the retina (see Figure 4). These components interact with each other and function as critical oscillators, but the relative contribution to the overt rhythmicity differs among species. Light can enter the avian circadian system through the retina, pineal gland, and deep brain photoreceptors (Gwinner & Brandstatter, 2001; Cassone, 2014).

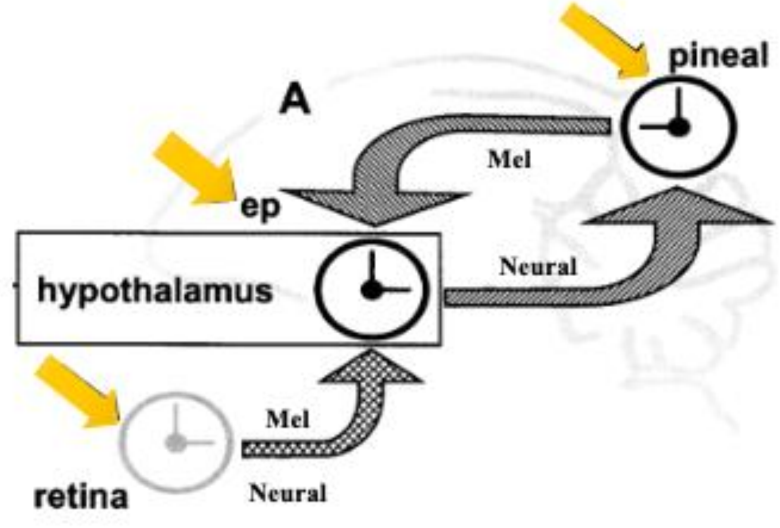


Figure 4: Diagram of the three main components of the avian circadian clock. Receive light input (yellow arrows). Ep = encephalic photoreceptors which is deep brain receptors. The oscillator interacts via either the hormone signal melatonin (Mel) or neural signalling pathways (figure slightly modified from Brandstatter, 2003).

1.2.2 Entrainment

In the presence of *Zeitgebers*, the circadian clock adjusts its period and phase to the environmental cycle. There are two main hypothesises explaining how environmental factors entrain the circadian clock: the parametric (“continuous”) and the non-parametric (“discrete”) entrainment model. In the parametric entrainment model the light intensity continuously modulates via advancing or delaying the speed of the clock, and focus on effect of the light duration (Aschoff et al., 1971; Daan, 2000; Roenneberg et al., 2010). In contrast, light in non-parametric model is proposed to cause a rapid change to the clock phase (phase shift), and focus more on the effect of the change in light intensity (Pittendrigh, 1960; Roenneberg et al., 2010). The magnitude of the phase shift depends on when the internal (“subjective”) circadian time is exposed to light. This can be explained by phase response curve (PRC), which is a plot of the magnitude and the direction of phase shift based on experiments where light pulses are given at different times (Pittendrigh, 1960). The main features of the PRC are the same for all species, with a phase-delay when light exposure are given around subjective dusk, while and phase- advance when it is given around subjective dawn. Light pulse given in mid-subjective day have no effect on the phase. These traits are important for entraining the circadian rhythm to the natural light-dark cycle (Pittendrigh, 1960; Roenneberg et al., 2010).

In addition, different oscillator models have been proposed to describe the entrainment to the natural light-dark cycle. The various output rhythms could be driven by one master oscillator, or could be the product of multiple, potentially independent, oscillators (Moore-Ede et al., 1982). Pittendirgh and Daan (1976) proposed a dual oscillator model ("two-oscillator model"), meaning that the circadian clock consists of two separate oscillators with different responses to light. A morning (M) oscillator, tracking dawn, accelerates in response to change in light intensity, and an evening (E) oscillator, tracking dusk, decelerates in response to changes in light intensity. The M and E oscillators are close to each other during short days and far apart during long days (Fig. 5A). This may be a flexible solution to an environment with changing photoperiod.

Entrainment consist of two equally essential concepts; the range of entrainment and the phase of entrainment, both dependent on the properties of *Zeitgeber* and the circadian clock (Moore-Ede et al., 1982; Schmal, 2020). The range of entrainment is the set of free-running periods that are able to entrain to *Zeitgeber* at a given strength and this range narrows or widens with decreasing or increasing *Zeitgeber* strength, respectively. The most important *Zeitgeber* to entrain the circadian clock to a 24-hour period in natural environment is the light-dark cycle (Moore-Ede et al., 1982). The ratio between light and dark depends on season and latitude, short photoperiod towards the winter solstice and long photoperiod towards the summer solstice. Individuals with varying free-running periods are able to entrain to the natural light-dark cycle with 1:1 light-dark ratio. However, during shorter or longer photoperiods, only a limited range of free-running periods can be effectively entrained giving rise to onion-shaped envelope (Fig. 5B; Schmal, 2020). In other words, an individual with a free-running period close to 24 hours are able to entrain to various photoperiods compared to those with shorter or longer free-running periods.

The second concept is the phase of entrainment, which ensure that physiological and behavioural processes occur at most beneficial times throughout the solar day. The stability of the phase relationship between an entrained rhythm and its *Zeitgeber* relies on both the period of the *Zeitgeber* and the free-running period of the endogenous oscillator driving the rhythm (Moore-Ede et al., 1982). If the free-running period is shorter than the period of the environmental cycle, the period of the endogenous oscillator must delay or extend to match the period of the environmental cycle, and the entrained rhythm will 'phase lead' (+ψ) the *Zeitgeber*. Conversely, if the free-running period is longer, the endogenous oscillator needs to advance or shorten the period, and the entrained rhythm experiences a 'phase lag' (-ψ) relative to the *Zeitgeber*. Alterations in the period of the *Zeitgeber* or free-running period can consequently impact the phase relationship (Moore-Ede et al., 1982). It is worth noting that the phase relationship between the *Zeitgeber* and the rhythm is also influenced by the choice of phase markers, as illustrated in Figure 5C.

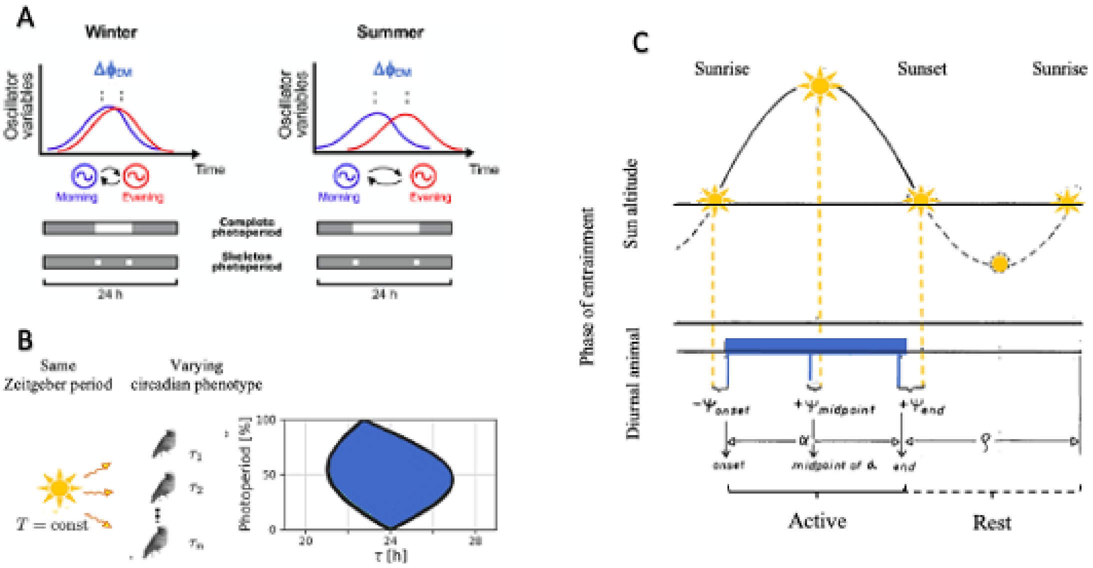


Figure 5: Concepts of entrainment to the light-dark cycle. A. Entrainment of the circadian clock to the natural light-dark cycle. A. Schematic illustration of the two-oscillator model, morning oscillator (M; blue) and evening oscillator (E; red), proposed of Pittendirgh and Daan (1976). M and E longer form each other during summer than winter (Figure slightly modified from Flôres et al., 2021). **B.** The range of entrainment in constant *Zeitgeber* period for different free-running period (tau, τ) with changing photoperiod (Figure modified from Schmal et al. 2020) **C.** Illustration of phase of entrainment (Figure modified from Daan and Aschoff, 1976).

The coupling strength between the *Zeitgeber* and the circadian rhythm is influenced by both the intensity of the *Zeitgeber* and the sensitivity of the endogenous oscillator to the *Zeitgeber*. A weak *Zeitgeber* may be insufficient to entrain the circadian rhythm, but able to modulate the period of the free-running rhythm in specific phases of the endogenous oscillator.

Resulting in a pattern of relative coordination where a rhythm is not entrained but still influenced by the environmental cycle (Moore-Ede et al., 1982). For example, if the change in light intensity of low amplitude coincides with the time of the “subjective dawn”, it may modulate the phase of rhythm and advance the circadian clock. However, it will not be enough to “phase-lock” the circadian rhythm with the low amplitude light cycle. This phenomenon can also be described by the PRC. Relative coordination to various weak *Zeitgebers*, e.g., light, temperature, social cue, or wheel availability, has been noted in different species (Erkert et al., 1986; Edgar & Dement, 1991; Emens et al., 2005; López-Olmeda & Sánchez-Vázquez, 2009). The environmental light cycle may also be too weak to affect the circadian clock at all, and the rhythm will free-run without relative coordination.

1.2.3 Output, and measuring biological rhythms

The circadian clock generates physiological and behavioural rhythms with an approximate 24-hour period, synchronising these patterns to the environment and giving rise to an entrained rhythm of 24 hours. Various rhythms have been measured to explore the inner timekeeping system, such as activity rhythms (e.g., locomotion, foraging, and the activity-rest cycle), physiological rhythms (e.g., body temperature or melatonin levels), and molecular rhythms (e.g., gene expression). To investigate if these rhythms are driven by an endogenous oscillator, experiments in constant condition such as constant darkness (DD) or light (LL) are necessary.

Nevertheless, evaluating the adaptive value of the circadian clock requires research in natural environments since animals in laboratory settings may exhibit behaviours different from those observed in the wild (Calisi & Bentley, 2009). This has been observed in several species of rodents. Examples of this phenomenon include the naked mole rat (*Heterocephalus glaber*) which, in captivity, displays short bursts of activity over a 24-hour cycle, whereas wild animals in their natural habitat exhibit temporally structured activity (Davis-Walton & Sherman, 1994; Urrejola et al., 2005). Other rodents are naturally diurnal but become nocturnal when placed in laboratory settings, such as the coruro (*Phyllotis darwini*; Urrejola et al., 2005), the unstriped Nile rat (*Arvicanthis niloticus*; Blanchong et al., 1999), and the tuco tuco (*Ctenomys coludo*; Oda & Valentinuzzi, 2023). The reason behind this shift in diurnal and nocturnal activity is largely unsolved, however the natural environment is very complex.

The correct timing of daily behaviour seems evident. It is presumed that the properties of the inner timekeeping system influence the timing of diel activity in the natural environment, potentially contributing to increased fitness (Johnson et al., 2004). As evolution have shaped circadian clocks in a cyclic world, it is more likely the mechanism of entrainment that has evolved and not the self-sustained rhythmicity in constant conditions (Roenneberg and Merrow, 2002; Johnson et al., 2004). Phase angle between circadian rhythm and the environmental cycle is influenced both by circadian properties as well as properties of the *Zeitgeber*, and therefore the phase angle can be altered by manipulating the free-running period and/or varying the period of the environmental cycle (T-cycles). It is hypothesized that having a free-running period closely aligned to the 24-hour day have an adaptive value, which may give rise to the optimal phase, however direct test remain limited. In a study, mice (*Mus musculus domesticus*) carrying a short-period mutation and wild-type mice were placed in outdoor enclosures. The mice with mutation had reduced survival rates when compared to their wild-type counterparts. These wild-type mice exhibited a circadian rhythm with a free-running period closely aligned with 24 hours (Spoelstra et al., 2016). This observation suggests the hypothesis that circadian rhythms with an endogenous period close to the natural light-dark cycle are evolutionary adaptive (Pittendrigh & Minis, 1972). However, potentially pleiotropic effects of the mutation may also have led to reduced survival of the mice carrying a short-period mutation.

1.3 Diel behavioural rhythmicity

Diel refers to rhythmicity with period of solar day that may or may not be driven by an endogenous oscillator. Diel behavioural rhythmicity is a widespread phenomenon observed in the animal kingdom. It is characterized by distinct patterns of activity and rest that often align with the natural light-dark cycle of the environment, and a seasonal change in activity pattern following the seasonal change in photoperiod (Fig. 6). While there is considerable variation among different species, some general trends can be observed. Most birds are diurnal, primarily active during the day, while most mammals species are nocturnal, active during the night (Bennie et al., 2014; Mukhin et al., 2009). Daily behavioural rhythmicity tends to be robust, but variations are noticeable across taxa. For instance, songbirds exhibit strong day-night rhythms, whereas species like geese and waders display weaker rhythms (Ebling & Piggins, 2020). It is important to note that behavioural rhythmicity can adapt to the environment and to specific behavioural contexts (Steiger, et al. 2013).

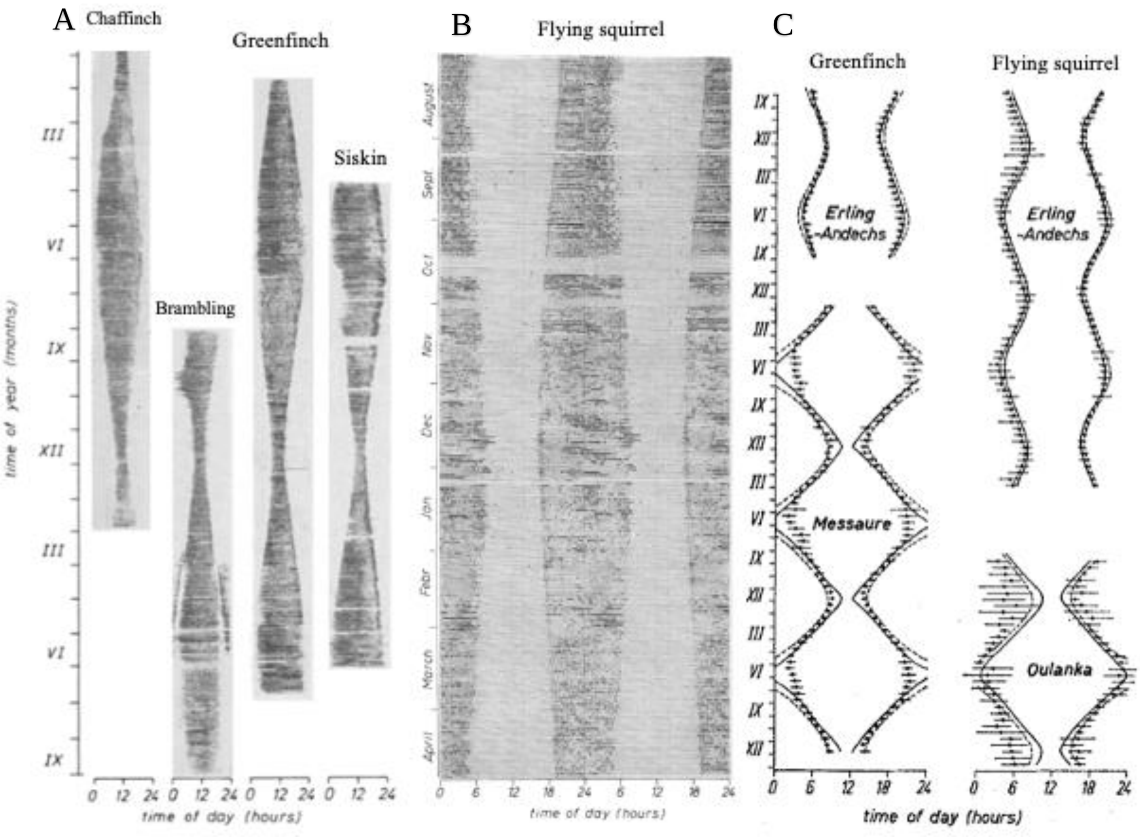


Figure 6: Annual activity recordings of different bird species and a mammal species at different latitudes. A. Bird activity records from Miessaure, Sweden (66.67ºN). **B.** activity records of flying squirrel at Erling-Andechs, Germany (47.97ºN). **C.** Seasonal change in the time of onset and offset daily activity for greenfinch (left) and flying squirrel (right) held in Erling-Andechs (top) and near the Arctic Circle (bottom). The solid lines indicate sunrise/sunset and the dashed lines indicate civil dawn/ dusk. The vertical lines indicate semi-monthly mean onset and offset, with a total 4 standard deviations (Figure slightly modified from Daan and Aschoff, 1976).

In natural environments and in the presence of cues such as the light-dark cycle, biological rhythms in animals can be determined by measuring activity rhythms, physiological rhythms, and molecular rhythms. Behavioural rhythms can be quantified given that the timing of the activities is repeatable and consistent. In birds, activities such as incubation (Bulla et al., 2016), foraging (Fitzpatrick, 1997), song (Naguib, et al., 2019) and sleep-wake cycle (Struber et al., 2015) are studied. Several approaches to monitor behavioural rhythms in wild animals exist, and there has been a rapid improvement in the ability to track animals in recent years (Dominoni et al., 2017). Example of methods used are radio frequency identification (RFID) (Bulla et al., 2016), GPS tracking device (Ensing et al., 2014), temperature and light loggers (Bulla et al., 2016), and camera traps (Surmacki & Podkowa, 2022; Schlindwein et al., 2023).

1.3.1 Chronotypes

Within a population, it is common to observe individual variations in the diel timing of behaviour. Individuals can be categorized into chronotypes, distinguishing between early or late individuals based on the timing of their activities relative to their counterparts. While chronotypes have been linked to performance in humans (Lim et al., 2021), in free-living species, the causes and consequences of inter-individual differences in diel timing remain poorly understood.

The chronotypes of the great tit, *Parus major,* and of the blue tit, *Cyanistes caeruleus,* both well-studied songbirds, have been shown to be repeatable (Graham et al., 2017; Womack et al., 2023). For male blue tits, individuals participating in the morning chorus earlier in the day demonstrated higher success in achieving extra-pair paternity, suggesting that variations in timing may be subject to natural selection (Poesel et al., 2006). Additionally, females initiating their activities earlier in the day started clutches earlier in the breeding season and raised more fledglings (Graham et al., 2017; Womack et al., 2023).

These variations in diel timing may be attributed to differences in clock properties, such as the free-running period or sensitivity to light (Helm and Visser, 2010; Womack et al., 2023). It is believed that individuals with different chronotypes exhibit variations in their free-running periods, with those having a shorter free-running period tending to be active earlier in the day than those with a longer free-running period. Domino et al., (2013) and Tomotani et al., (2023) demonstrated such relationship between the free-running period and diel timing of activity for great tits. However, Helm and Visser (2010) did not find such relationship between the free-running period and chronotype. Overall, the differences in diel timing can be considered a proxy for differences in endogenous rhythms, as the phase angle between the *Zeitgeber* and the timing of activity may depend on an individual's free-running period (Roenneberg et al., 2003). Chronotype, which reflects the individual’s phase of entrainment under the same light-dark cycle, can change with varying *Zeitgebers*. Moreover, chronotypes may change with life history, age, and show variations between sexes, as seen in humans (Fischer et al., 2017), and animals such as great tit where males start their activity before females (Kluijver, 1950).

1.4 Diel behavioural rhythmicity in the Arctic

Research on arctic animals, such as Svalbard ptarmigan (*Lagopus muta*) and Svalbard reindeer (*Rangifer tarandus platyrhynchu*), has led to the speculation that a weakened circadian clock is beneficial in the Arctic. They have shown diurnal activity in the presence of light-dark cycle, but the rhythmicity abolishes during constant conditions (Stokkan, Sharp et al. 1986; Reierth and Stokkan, 1998; van Oort, Tyler et al. 2005, 2007). These results suggest that diurnal activity during light-dark cycle is a passive response to the environmental cycle rather than driven by an endogenous oscillator. A more recent study also suggests adaptive value of periods with arrhythmicity of polar animals, where arctic charr (*Salvelinus alpinus*) showed arrhythmicity during polar day (Hawley et al., 2017). Activity around-the-clock and arrhythmicity may be advantageous traits for animals living in weak rhythmic environment such as the Arctic. These traits may represent a weakened circadian clock or circadian clock uncoupled from its output (Bertolini et al., 2019). Diurnal animals able to take advantage of the continuous daylight during polar day for activities such as foraging, and therefore not missing food opportunities, may be favourable. In contrast, polar night, challenges diurnal animals, as it can lead too little time to build up food reserve to survive the long nights and winter (the opposite for nocturnal animals) (Hazlerigg et al., 2023).

However, other animals resident in the Arctic exhibit diel activity rhythms under polar day and night and, therefore, do not support this speculation. Some examples are the Arctic ground squirrel (*Urocitellus parryii*), in Northern Alaska (68 ^{o}N), which displayed diel body temperature during 6 weeks of polar day (Williams, Barnes et al., 2011), and the bumblebee (*Bombus terrestris*) population in northern Finland (69 ^{o}N) which had a robust diel foraging rhythm during polar day (Stelzer and Chittka, 2010). Another study on free-living polar bears (*Ursus maritimus*) shows diel activity rhythmicity both during polar day and polar night, although interindividual differences were reported (Ware et al., 2020). Furthermore, a study on several arctic-breeding birds shows different responses during polar day. Depending on species, sex, and breeding stage show either arrhythmicity, entrainment or “free running” patterns (Steiger, et al. 2013).

Overall, there is wide variation in behavioural patterns in animals during polar day and/or night. Some species maintain a diel activity pattern, whereas other become arrhythmic. The animals response to a reduction in the amplitude of environmental cycles, such as the natural light-dark cycle, provides valuable insights into their circadian clocks, as behavioural rhythms are often outputs of the inner timekeeping system. Figure 7 illustrate four distinct scenarios of activity patterns during continuous daylight, such as during polar summer, based on experiments of circadian clock and observation of diel behavioural patterns in wild animals in their natural environment. It is possible that they are still entrained by the low amplitude solar cycle (or other weaker *Zeitgebers* than the natural light-dark cycle), start to free-run when the *Zeitgeber* is too weak (or relative coordination), the diel activity rhythm breaks down over time, or the loss of rhythmicity when there is no clear day and night as the sun is continuously above the horizon. These difference in behavioural patterns may suggest differences in circadian clock properties, and an adaption to the arctic light regime where different species may have different strategies. However, in order to state if the behavioural activity pattern observed in wild animals is driven by the inner timekeeping system it would be necessary to conduct more experiments in constant conditions.

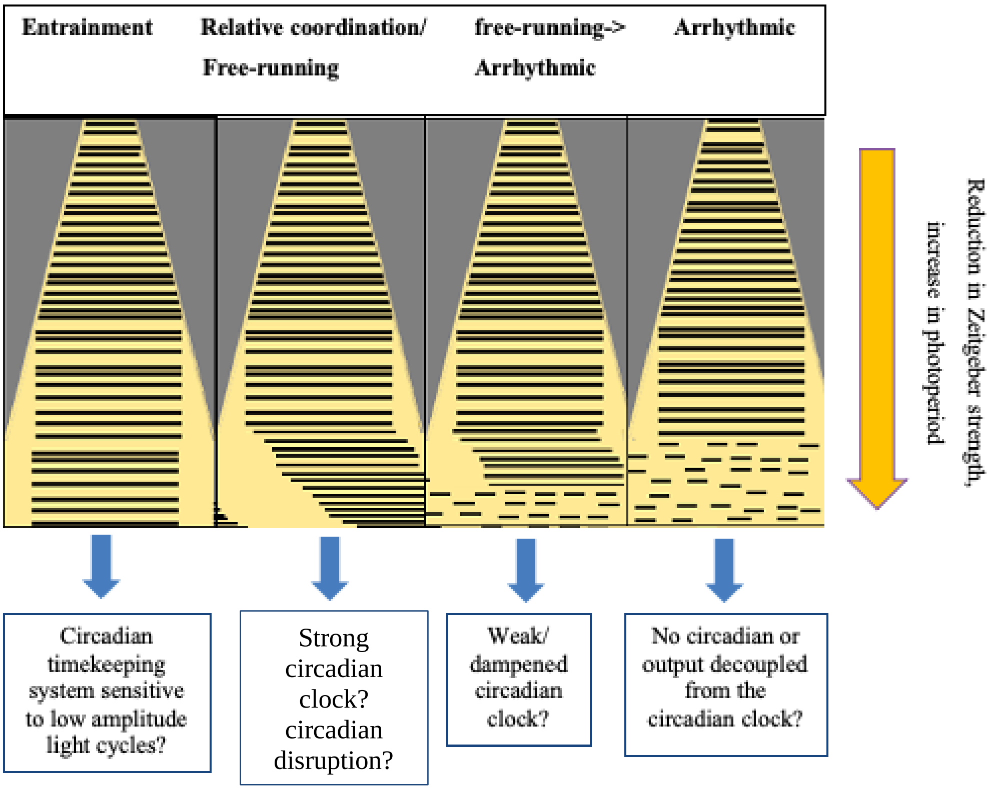


Figure 7: Schematic illustration of four distinct scenarios of activity patterns with the reduction in *Zeitgeber* strength and increase in photoperiod, and what it may suggest about their inner timekeeping system.

1.4.1 Colonization of Arctic regions by southern species

Over the past few decades, our planet has undergone global warming, a trend that shows no signs of reversing. With the climate change, organisms shift their geographical distribution where Southern species colonize Northern areas and are, consequently, exposed to novel light conditions (Pakanen et al., 2018). The introduction of novel light, such as irregular light-dark patterns or light exposures at the “wrong” circadian time (internal “subjective” time), as seen in night shift workers, can disrupt circadian organisation. This exposure result in a misalignment between the inner timekeeping system and the natural light-dark cycle, potentially leading to a state of internal desynchronisation. Disruption of the circadian organisation have been associated with adverse effects on sleep patterns, hormonal regulation, and mental health in humans (Boivin et al., 2021).

The properties of the circadian timing system are anticipated to exhibit systematic variations with latitude, driven by the distinct annual light and temperature patterns at higher latitudes, imposing specific selection pressures (Hut et al., 2013). Consequently, as Southern species colonize Northern areas, we expect the inner timekeeping system to undergo selection pressures, shifting towards weakened circadian system that may be advantageous in a weak rhythmic environment. Despite these expectations, research on the diel rhythmicity and circadian clocks of southern species that have colonised the Arctic regions remains limited. A study on high and low latitude species of drosophilids reveals that the high latitude species lose rhythmicity when exposed to constant darkness. Their circadian clock exhibits signs of either not being self-sustained or becoming decoupled from its output (Bertolini et al., 2019). This finding suggests that the capacity to maintain behavioural rhythms may have undergone multiple evolutionary losses during the evolution of drosophilids, potentially representing an adaptive response to life at high latitudes (Bertolini et al., 2019).

1.5 Significance and purpose

Global warming drives southern species to move to higher latitudes, and consequently encounter novel light conditions which raises the questions: Do the novel light conditions disrupt their circadian organisation and timing of behaviour? Are they able to adapt to the novel light conditions, and if so, how fast? Studying the daily behavioural rhythms in southern species that have colonised the Arctic can provide us with more information to answer these questions. The great tit is a common study species, and increasing the number of studies to include populations at higher latitude can further help examine the correlation of biological rhythms with the environment. This can be done by studying biological rhythms in the same species at different latitudes. As well as the close related songbirds, blue and willow tits, which have colonised Northern areas at different times (Pakanen et al., 2018), can give more insight on adaption to novel light conditions.

The aim of this study is thus to investigate and characterise the daily behavioural rhythmicity of wild songbirds inhabiting the Arctic region, across seasons on a daily and annual scale, thereby encompassing a wide range of light conditions, including the polar nights and polar days. By comparing three songbird species—great tit, blue tit, and willow tit—belonging to the Paridae family, this can give an insight on the rhythmic patterns exhibited by non-Arctic species that have colonised the Arctic environment.

Main Objective: Characterize diel activity pattern in three species of wild songbirds in the Subarctic on population level throughout the year.

Specific objectives:

- Does the activity pattern of three bird species differ compared to each other?
- Does the activity pattern change during the year in response to different light conditions?
- Is it possible to detect individual differences?

2 Material and Methods

2.1 Study site

Daily activity data of great tit, blue tit and willow tit was collected at Tromsøya (69° 39′ N, 18° 57′ O). 89 nest boxes and 6 feeders were placed, however in thesis I used activity data from three feeders (see Fig. 8, and see Appendix A for the complete study site). The three feeders were placed near Tromsø university and placed approximately 250 – 350 m apart each other (Fig. 8). The study site consists patches of mixed forest dominated by birch (*Betula pubescens*). The data collection period spanned from 13 November 2022 to 1 July 2023. Polar twilights last from 28 November to 15 January, and the period with polar days started on 18 May and ended on 26 July. In 2023, Tromsø was covered by snow from the beginning of December to mid of May. The coldest day was 7 March (mean temp: -10.1 °C) and the warmest day was 22 June (mean temp: 20.1 °C)

Figure 8: Map of the study site Tromsø, Norway with the location of the three feeders. Map provided from kartverket (2023).

2.2 Study species

There was a high diversity of bird species visiting the feeders. However, in this study we focused on three tit species, the great tit (*Parus major*), blue tit (*Cyanistes caeruleus*), and willow tit (*Poecile montanus*) due to their consistency in timing and commonly used in research. These birds exhibit distinct differences in appearance, which makes it easy to identify them (see Figure 9). Great and blue tits occupy range of different habitats, such as open deciduous woodland, mixed forests, forest edges, parks and gardens. While willow tits occupy wet woodlands and coniferous forests. Great and blue tits are cavity nesters, they build nest in small holes in tree trunks, while willow tits normally excavate their own nest holes. They are great study animals in the wild because they commonly use feeders, and great tits and blue tits frequently use nest boxes which make it relatively easy to collect behavioural activity in the wild. Great and blue tit populations are partially migratory, which made it possible to observe their activity during the cold subarctic winter.

Figure 9: Pictures of the three bird species, great tit (left), blue tit (middle) and willow tit (right).

2.3 Activity recordings

To investigate the daily activity of wild songbirds, we utilized a combination of feeders and camera traps, with daily visits to the feeder serving as a proxy for measuring daily activity. At each study site, a singular feeder with multiple chambers, featuring either 2 (small) or 4 (large) chambers, was placed (Fig. 10). However, only two were accessible at any given time—one on each side. The feeder was attached to a tree (later, also secured to the ground for enhanced stability). The feeders were filled 1-2 times weekly with sunflower seeds. Once the birds started to use the feeders, a camera trap (Strike Force Pro X) equipped with infrared night vision was positioned on a nearby tree or branch, maintaining an approximate distance of 40 cm from the feeder. The camera was oriented to ensure both feeder openings were visible in the captured images. In order to capture intricate details, custom-made lenses were integrated into the camera setup, allowing us to obtain good quality photos at a closer range. The camera operated continuously throughout the day, utilizing a motion detection interval of 5 seconds. Local wintertime was used during the whole study period.

For Feeder 1 and Feeder 2, modifications were made, transforming them into feeder boxes. The purpose of this modification was to enhance the quality of images, resulting in better focus and increased resilience towards weather and wind conditions. The modification was also done for the purpose of bird ringing, henceforth referred to as trap box (Fig. 10). The quality of the images improved considerably. However, this modification led to high diversity of birds visiting the trap box, consequently, displaced the tits. Therefore, we decided to return to the original feeder at site 1. The three feeders were placed and taken down at different times, as outlined in Table 1.

Table 1: The time of camera Attachment and the change of feeder types. Feeder 1 and 2 was running to the end of the study period.

Site	Start	Ended	Type
Feeder 1	13.11.2022	07.02.2023	Feeder → Trap box → Feeder
Feeder 2	16.02.2023	07.02.2023	Feeder → Trap box
Feeder 3	03.03.2023	22.05.2023	Feeder

Figure 10: The set-up of feeder with camera trap. Left: Small feeder with two chambers and camera trap. Right: Large feeder modified to a feeder box with camera trap, referred as trap box. With two great tits inside (Right picture: B. Tomotani).

2.3.1 Individual activity data

To quantify individual daily activity, birds were individually ringed using metal rings obtained from the Norwegian Bird Ringing Centre at Museum Stavanger. Furthermore, each bird was ringed with a distinctive colour ring, enabling easy differentiation between individuals. In the case of great tits, an additional black ID ring with a unique number (e.g. A01) was applied. The first birds were ringed March 19th and the last was ringed May 24th. Throughout the study period, a total of 16 tits were ringed, comprising of 9 great tits, 2 blue tits, and 5 willow tits (see the Appendix B, Table B-1 for detailed information).

Capture was conducted using two different methods: the utilization of mist nets and trap boxes. Prior to their release, the birds were weighed, and sex and age were determined to the extent possible. Ringing birds requires a licence, therefore, this process was carried out by my supervisor Barbara Tomotani.

2.4 Data extraction

Images were collected once to twice per week, mainly during midday or in the afternoon to minimize disturbance at the start and end of their activity. All collected images were stored on Dropbox initially and later transferred to an external hard disk. To process the large volume of camera trap images efficiently, we used SpeedyMouse 2.2 (Spoelstra, 2016) for species and individual scoring. For great tits, scoring included categories like ID or unringed, female, or male where applicable (see Appendix B to see how we scored the birds). Sex determination based on camera trap images were only possible for great tits due to a thicker belly stripe on males compared to females.

Environmental data

Environmental factors, such as light intensity, were not directly measured in this study. Instead, daily timing data for sunrise, dawn, sunset, and dusk were sourced from timeanddate.no. Additionally, sun position parameters, including altitude and azimuth angles, were extracted from the same source. Temperature data were obtained from yr.no.

2.5 Data analysis

Chronoshop 1.1 (Spoelstra, 2016) was used for analyses of the activity data. Chronoshop was used to determine onset and offset of activity. The onset of activity was calculated for each cycle by going 0.5 cycle back in time from the center of gravity (CoG). Offset of activity was found the same way by going forward 0.5 cycle from the CoG. The CoG was positioned at the mean vector angle, calculated by averaging the sine and cosine values of activity within one cycle, using the formula:

$$\mathrm{CoG}_{(x,y)} = \left(\frac{\sum_{i=1}^{n} y_i \sin\left(\frac{2\pi i}{n}\right)}{\sum_{i=1}^{n} y_i}, \frac{\sum_{i=1}^{n} y_i \cos\left(\frac{2\pi i}{n}\right)}{\sum_{i=1}^{n} y_i} \right)$$

The running mean was configured with a window size of 1 bin to ensure accurate activity detection when a bird was present at the feeder. A bird was considered active when it was detected at the feeder.

2.5.1 Data cleaning

In this thesis, feeder visits serve as a proxy for activity. The frequency of visits to the feeders varied throughout the study period, with some days with numerous visits, while others had comparatively fewer. This resulted in instances where the first registered visit at the feeder occurred relatively late in the day, and/or the last registered visit was relatively early. Days with minimal visits may not accurately represent bird activity. To ensure the reliability of our statistical analyses, we excluded certain days that exhibited a late onset or early offset of daily activity—deviating from normal and expected patterns—or had limited available activity data. Data cleaning involved a manual inspection of the actogram generated in Chronoshop, along with examination of phase marks such as onset and offset (Fig. 11). Each day within the study period for great tit, blue tit, and willow tit was examined manually to determine whether the data appeared accurate or not. Days were excluded from statistical analyses and not considered as proxies for the onset and offset of activity if the first and last registered visits at the feeder deviated from those of the days before and after.

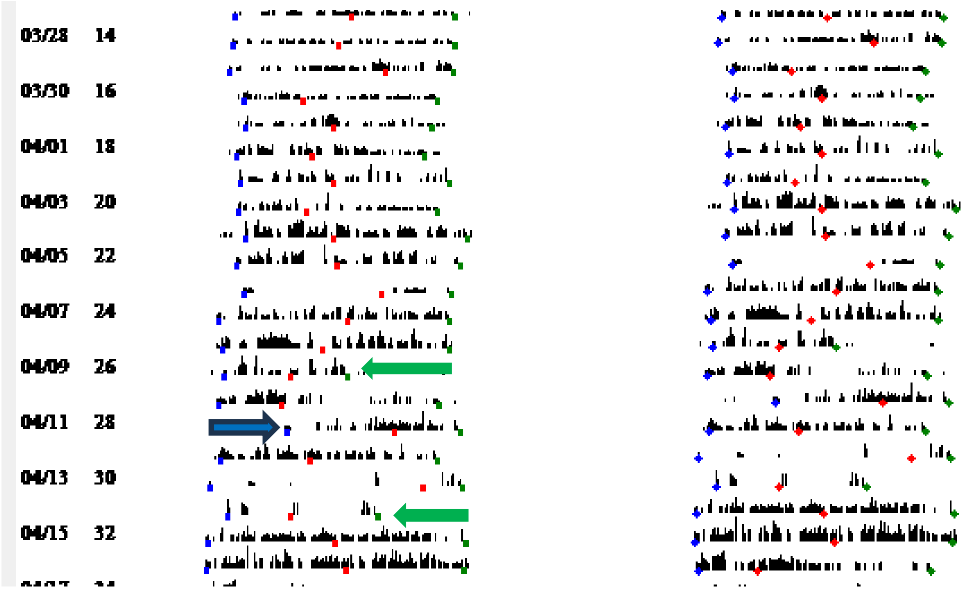


Figure 11: Double-plotted actogram generated in Chronoshop with phase marks onset (blue), offset (green) and CoG (red). Blue and green arrows indicate days with onset and offset from the normal.

Number of days not included in the data analyses of:

Great Tit:

- Removed the last 11 days of the study period due too little to no activity data. In addition, 20 days were excluded from the analyses.

Blue Tit:

- Removed the last 17 days due to no data or small amount of data, and 37 days were excluded from the analyses.

Willow Tit:

- 52 days were excluded from the analyses.

2.6 Statistical analyses

Actograms and illustration of annual onset and offset of activity were made in Excel version 16.80. The rest of the plots and all statistical tests were made and performed in R version 4.2.2 (R Core Team, 2022). Multiple linear regression models were used to analyse the relationship between activity, seasons, and temperature for the different species. Thereafter, Tukey post hoc tests was used to compare the daily activity pattern between species at same season and between season for the same species using the *emmeans* library in R (see Appendix G for the R-codes). The Holm method was employed to adjust p-values for multiple comparisons, to control for the overall Type I error rate (Aickin & Gensler, 1996). The null hypothesis (H0): all means are equal to each other ($\mu 1 = \mu 2 \ldots = \mu_i$) was not rejected when $p > 0.05$ or close to this threshold. Backwards model selection was used to obtain p-values and find the best fitting model by dropping single non-significant variables every step ($p > 0.05$) using the drop1 function and F test in R. Estimates were extracted from the model with only significant interactions.

2.6.1 Analyses annual changing onset and offset of activity

To analyse changes in the onset and offset of activity, the study period was segmented into five distinct seasons based on photoperiod and the time of year (see Table 2). Polar twilights and polar days were treated as separate seasons, labelled DD, and LL, respectively. Within the light-dark cycle periods, segmentation was applied to represent their respective seasons autumn, winter and spring. The time before polar twilight was designated as LD autumn, while the period from polar twilight to the end of winter months was categorized as LD winter. The interval from spring until polar days was defined as LD spring.

Table 2: Outline the specific dates and corresponding to each season.

Season	**Date**
LD autumn	13.11 – 28.11
DD (polar twilight)	29.11 – 15.01
LD winter	16.01 – 28.02
LD spring	01.03 – 18.05
LL (polar days)	19.05 – 01.07

There were instances where the time of activity onset were before midnight which lead to activity spanning over two days. To accommodate for this, I added 5 hours to each onset time, thereby maintaining the relationship between the onset timepoints, while ensuring that all timepoints fell within the same day. This adjustment was made before conducting the multiple linear regression analyses. To obtain accurate estimates, I subsequently subtracted 5 hours, aligning the data with the original time frame.

For the onset analyses, the response (dependent) variable was onset of activity, and the predictor (independent variables) were seasons, species, average temperature and the interaction between season and species. The model for offset was similar to the onset model, except average temperature did not have a statistically significant effect on offset of activity and was therefore excluded from the model.

Full model used for the analyses of onset:

Onset ~ Species: Season + Species + Season + average.temp

Full model used for the analyses of offset:

Offset ~ Species: Season + Species + Season

Thereafter, Tukey post hoc tests were used to investigate if and which species and seasons differ from each other.

2.6.2 Analyses of Activity time

In order to analyse the activity-rest cycle I used activity duration. The nocturnal activity during polar twilight were excluded from the analyses of activity. Activity duration was calculated by subtracting the onset of the previous day from the offset of the current day:

Activity time = offset of the current day – onset of the previous day

When the activity started previously day (before midnight) 24 was added to the equation:

Activity time = (offset of the current day – onset of the previous day) + 24

Thereafter, multiple linear regression models were used fitting activity time as the dependent variable and species, season, average temperature and the interaction between species and season as independent variables to investigate.

Activity time ~ Species: Season + Species + Season + average.temp

Thereafter, a Tukey post-hoc test was used to do pairwise comparisons between seasons and species.

2.6.3 Polar days and polar plots

Animals may be entrained by other light cues than the light-dark cycle during polar days, such as the position of the sun. However, it is not clear what to use as phase markers for that type of *Zeitgeber*. Thus, to visualize this data, polar plots were made in R studio using the coord_polar function in the *ggplot* library. The number of visits to the feeders was summed per hour for each species at population level. Azimuth and time were placed on the polar axis.

2.6.4 Analyses of periodicity

The period of the activity rhythms were only calculated the last part of the study period as we only had individual activity data from mid-March. The period of activity was estimated using the Lomb-Scargle periodogram (Ruf, 1999) performed in ImageJ plugin ActogramJ (Schmid, Helfrich-Förster et al. 2011) for 15-day intervals for individuals. The normalized power index (PN) is calculated based on the following formula:

$$PN = \frac{1}{2\sigma^2}\left\{\frac{\left[\sum_{i=1}^{N}(x_i - M)\cos\left(\frac{2\pi}{P}(t_i - \delta)\right)\right]^2}{\sum_{i=1}^{N}\cos^2\left(\frac{2\pi}{P}(t_i - \delta)\right)} + \frac{\left[\sum_{i=1}^{N}(x_i - M)\sin\left(\frac{2\pi}{P}(t_i - \delta)\right)\right]^2}{\sum_{i=1}^{N}\sin^2\left(\frac{2\pi}{P}(t_i - \delta)\right)}\right\},$$

M = mean of all N values

σ^2 = Variance of all N values

P = Period being tested

δ is used to adjust the times of unequally spaced data and defined as:

$$\delta = \frac{1}{\frac{4\pi}{P}}\tan^{-1}\left[\frac{\sum_{i=1}^{N}\sin\left(\frac{4\pi}{P}t_i\right)}{\sum_{i=1}^{N}\cos\left(\frac{4\pi}{P}t_i\right)}\right]$$

The algorithm calculates PN across a defined range of period lengths, here set from 16 to 32 hours. The true period is determined as the value with the highest PN with a significant p-value under 0.05. We used the Lomb-Scargle periodogram because this method is particularly suited to detect periodicity in unequally sampled time-series and data sets with missing values. This method was well-suited for our activity data, given the large variation of visits per day and the presence of missing values.

The average period for the “nocturnal activity pattern” observed in the great and blue tit population during polar twilight was manually calculated based on the time of onset:

$$\text{Period} = Z - \frac{\sum_{i=1}^{N}(Onset_{i-1} - Onset_{i})}{N}$$

Z = period of the Zeitgeber

$Onset_{i-1}$ = previously recorded onset time

$Onset_{i}$ = onset time at day i

N = number of days

2.6.5 Calculation and analyses of phase angle differences

With the annual change in light conditions, we would expect that onset and offset of activity would also change accordingly. To explore the relationship between activity patterns and changing photoperiod, we conducted an analysis involving the calculation of phase angle differences between the phase of the *Zeitgeber* and the phase of the activity rhythms. Diurnal animals’ onset and offset of activity often follows the civil dawn and civil dusk, respectively (Daan and Aschoff, 1976). In addition to calculating the phase angle differences between civil dawn and onset of activity, and civil dusk and offset of activity sunrise/ sunset, were used as phase marks. We used also midpoint of activity as phase mark for the activity rhythm, and midpoint of the solar day using sunrise to sunset, and also from civil dawn to civil dusk. Phase-angle differences Ψ (in hours) was calculated for the three species for the following parameters:

Ψ-Onset = Sunrise – Onset,
Civil dawn – Onset,

Ψ - Offset = Sunset – Offset,
Civil dusk – Offset

Ψ - Midpoint = 0.5 * (Ψ- Onset + Ψ-Offset)

Activity onset is phase-leading the light-dark cycle if the time they start their activity before the time of sunrise, the same for midpoint and offset daily activity. In contrast, phase-lagging if the time of either onset, midpoint or offset is after sunrise, midpoint of the light-dark cycle or sunset, respectively.

The nocturnal activity during polar twilight were excluded from the analyses of phase angle differences between the phase marks onset and civil dawn. In addition, the phase angle differences between offset and civil dusk for blue tit during polar twilight (DD) were excluded from the analyses of Ψ - Offset. For the analyses of Ψ - Midpoint both nocturnal activity and the Ψ - Offset for blue tit during polar twilight (DD) were excluded.

Thereafter, these parameters were fitted into multiple linear regression models as the dependent variables. For the analyses of Ψ-Onset both using sunrise and civil dawn as phase marks for the *Zeitgeber*, the independent variables were seasons, species, average temperature and the interaction between season and species.

Full model used for the analyses of Ψ-Onset:

Ψ-Onset ~ Species: Season + Species + Season + average.temp

For the analyses of Ψ-Offset using sunset as phase marks for the *Zeitgeber*, the independent variables were species, season and average temperature. Whereas, for the analyses of Ψ-Offset using civil dusk as the phase mark the independent variables were species, season, average temperature and the interaction between species and season.

Full model used for the analyses of Ψ-Offset with sunset as phase mark:

Ψ-Offset ~ Species + Season + average.temp

Full model used for the analyses of Ψ-Offset with civil dusk as phase mark:

Ψ-Offset ~ Species: Season + Species + Season + average.temp

For the analyses of Ψ - Midpoint using midpoint of light-dark cycle from sunrise to sunset, the independent variables were species, season and average temperature. Whereas, for the analyses of Ψ-Midpoint using midpoint of light-dark cycle from civil dawn to civil dusk, the independent variables were species, season and the interaction between species and season.

Full model used for the analyses of Ψ-Midpoint (sunrise to sunset):

Ψ - Midpoint ~ Species + Season + average.temp

Full model used for the analyses of Ψ-Midpoint (civil dawn to civil dusk):

Ψ - Midpoint ~ Species: Season + Species + Season

Thereafter, Tukey post hoc tests were used to investigate if and which species and seasons differ from each other for all the parameters listed above.

3 Results

3.1 Annual activity rhythms

During spring and autumn, when the birds were exposed to a light-dark cycle (sunrise and sunset) all three species exhibited a diurnal activity pattern. Remarkably, none of the feeder-visiting birds lost their rhythms when the natural light-dark cycle was absent (during the polar summer and winter). Notably, only the great tit and the blue tit displayed nocturnal activity during polar twilight and during the polar day (Fig. 12).

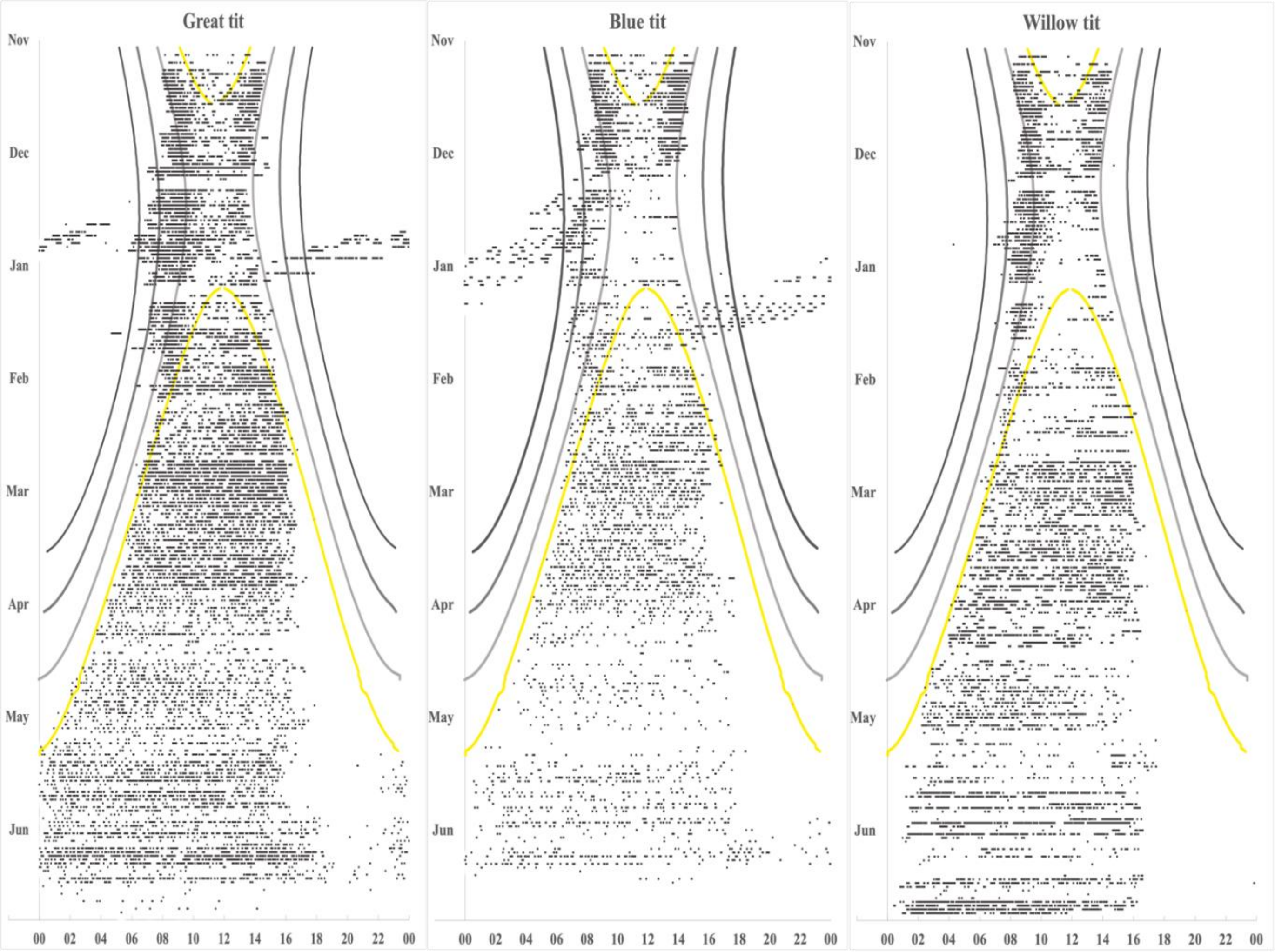


Figure 12: Actograms illustrating activity recordings, from camera-traps located beside feeders, of great tit (left), blue tit (middle) and willow tit (right). The black “dots” indicate activity. Time of year in months is illustrated on y axis and time of day (hour) on x axis. The grey lines indicate start and end of twilight phases: Astronomical twilight (dark grey line), nautical twilight (grey line), and civil twilight (light grey line), and sunrise or sunset (yellow line).

3.1.1 Onset and offset of activity

The seasonal course of onset and offset of daily activity of great tit, blue tit, and willow tit population (Fig. 13) shows that the activity time do not follow the seasonal changes in duration of the day in any of the three species. The time of onset of activity changes throughout the year with the changing light-dark cycle. On the other hand, offset of activity did not track sunset or civil dusk for most of the year.

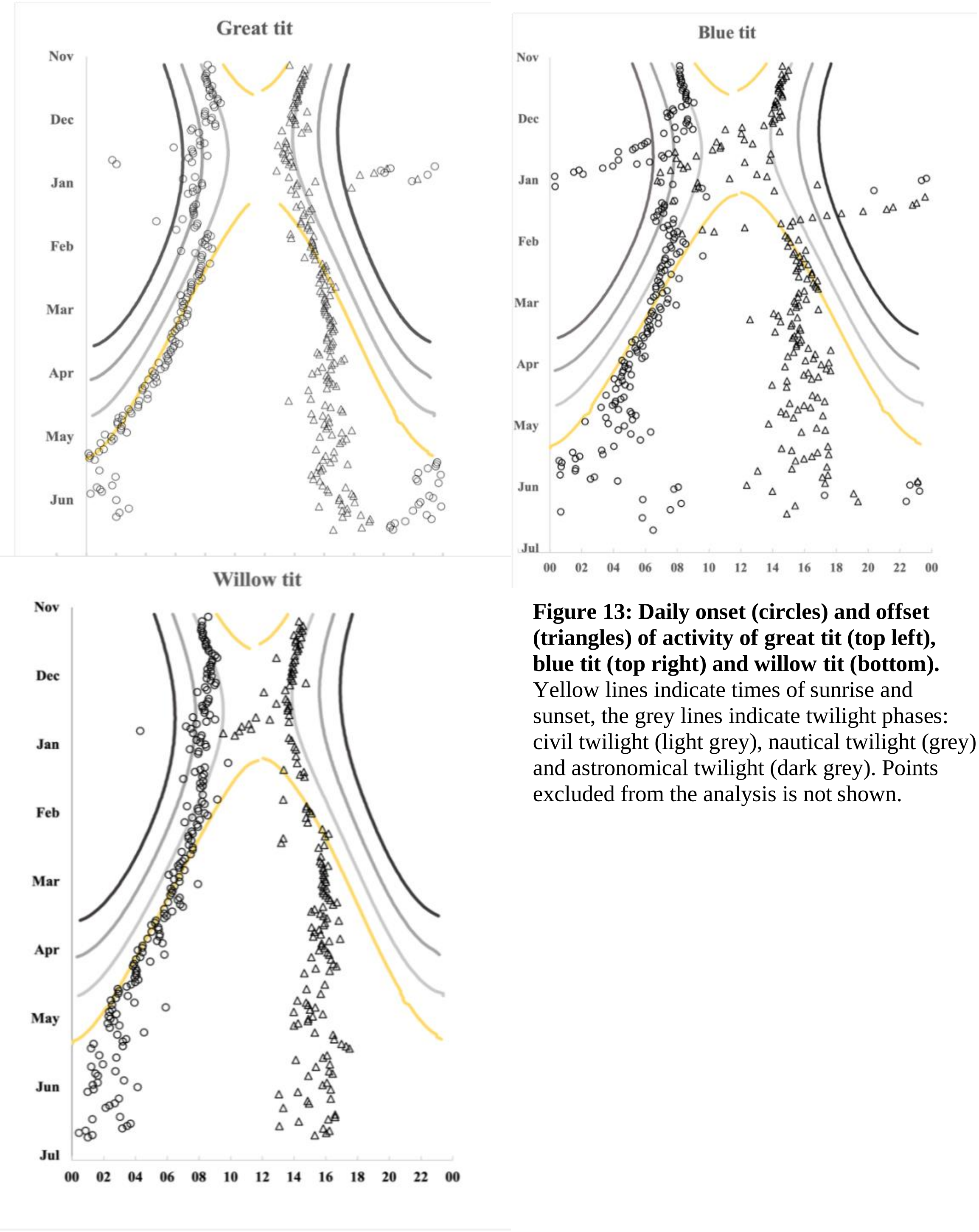


Figure 13: Daily onset (circles) and offset (triangles) of activity of great tit (top left), blue tit (top right) and willow tit (bottom). Yellow lines indicate times of sunrise and sunset, the grey lines indicate twilight phases: civil twilight (light grey), nautical twilight (grey) and astronomical twilight (dark grey). Points excluded from the analysis is not shown.

The time of onset and offset of activity in local time change within season for each tit species. The interaction between species and season was statistically significant for both onset and offset of activity (Onset: F = 8.70, p < 0.001, offset: F = 7.58, p < 0.001). The time of onset changed more with season starting earlier towards summer, whereas, the offset of diel activity changed less (Table 3). For the analyses of onset, we excluded the nocturnal activity for great tit and blue tit as it affects the result (marked with orange in the Table 3). Temperature had only statistically significant effect of time of onset (F =125.89, p<0.001).

Table 3: The onset and offset estimates and standard error (in minutes) for great, blue and willow tit during the different seasons. The times are in local wintertime.

Season	**Great tit**		**Blue tit**		**Willow tit**	
	Onset	**Offset**	**Onset**	**Offset**	**Onset**	**Offset**
LD autumn	07:58 +/- 68	14:23 +/- 96	08:02 +/- 38	14:34 +/- 34	08:05 +/- 68	14:05 +/- 97
DD	07:11 +/- 11	14:26 +/- 16	07:45 +/- 14	12:29 +/- 17	07:59 +/- 10	12:53 +/- 16
LD winter	07:20 +/- 51	15:16 +/- 72	07:13 +/- 32	16:34 +/- 24	07:50 +/- 51	13:36 +/- 75
LD spring	04:30 +/- 48	16:14 +/- 66	05:24 +/- 30	15:43 +/- 22	04:48 +/- 48	15:39 +/- 66
LL	00:22 +/- 61	16:08 +/- 84	02:16 +/- 37	16:54 +/- 47	03:08 +/- 61	15:56 +/- 85

Nocturnal activity was observed for great and blue tits during polar twilights (Table 4). Nocturnal activity was first observed 1 month into the period of polar twilight for great tit, while for blue tit nocturnal activity were observed 7 days earlier than great tit. The estimated average periods of the “nocturnal activity pattern” were 22.37 h for great tit, and 23.34 h for blue tit (Table 4).

Table 4: Observed onset of diel activity during polar twilight for great and blue tit. Date and time for the first observed onset of nocturnal activity and onset before midnight during polar twilight, and the period of the free-running activity pattern. The times are in local wintertime.

Season	**Great tit**	**Blue tit**
DD (polar twilight)		
Nocturnal activity	Dec 29th 01:44	Dec 22st 05:57
Before midnight	Jan 1st 23:26	Jan 7th 23:39
Average Period	22.37 h	23.34 h

Great tit and blue tit start again their activity before midnight when the natural light-dark cycle is absent during summer (Fig. 12 and 13). All three tit species initiate their activity earlier compared to the spring season ($p < 0.001$; see Table 5). On average, blue tits started their activity 3.1 hours earlier during polar day than in spring, while great tits initiate activity 4.1 hours earlier, and willow tits start 1.7 hours earlier during polar day compared to spring.

Great tit started in average 22 minutes after midnight, blue tit started their activity in average 2 hours and 16 minutes after midnight, and willow tit started activity in average 3 hours and 8 minutes after midnight. Furthermore, Tukey post hoc test revealed statistically significant differences of the time of onset of activity between all the species combinations, blue tit and great tit (mean difference: 1.91 h, $p <0.001$), blue tit and willow tit (mean difference: -0.86 h, $p =0.018$), and great tit and willow tit (mean difference: -2.76 h, $p<0.001$; Table 6). In contrast, it was only a statistically significant difference between the time of offset during spring compared to offset during polar days for blue tit (mean difference: -1.18 h, $p = 0.036$; Table 5). Additionally, there were no difference between the species in the time of offset during polar days (see Appendix D).

Table 5: Tukey post hoc – Differences in the time of onset and offset (local wintertime) between LD spring and polar day (LL) for each species. Diff = season 2 – season 1, and SE are given in hours.

	Season 1	Season 2	Diff	SE	t-ratio	p-value
Blue tit						
Onset:	LD spring	LL	3.12	0.33	9.490	<.0001
Offset:	LD spring	LL	-1.18	0.45	-2.621	0.0361
Great tit						
Onset:	LD spring	LL	4.13	0.27	15.315	<.0001
Offset:	LD spring	LL	0.10	0.37	0.283	1.000
Willow tit						
Onset:	LD spring	LL	1.67	0.28	5.898	<.0001
Offset:	LD spring	LL	-0.28	0.40	-0.702	0.8453

Table 6: Tukey post hoc - Interspecies differences in the time of onset in local time during polar day (LL). Diff = Species 2 – Species 1. Diff and SE given in hours.

Species 1	Species 2	Diff	SE	t-ratio	p-value
Season: LL					
Blue tit	Great tit	1.91	0.36	5.363	<.0001
Blue tit	Willow tit	-0.86	0.36	-2.358	0.0187
Great tit	Willow tit	-2.76	0.31	-8.953	<.0001

3.2 Activity time

The statistical analysis revealed a significant interaction between species and season for activity time (F = 7.51, p < 0.001). Activity duration increased progressively towards summer for all species. There was a statistically significant difference between both species and season (Fig. 14). There were no statistically significant differences between activity time between autumn, polar twilight and winter (p>0.05). The tit populations were active for approximately 6 – 7 hours during autumn and polar twilight (Table 7). There were statistically significant differences in activity time for all species between LD winter and LD spring, and between LD spring and LL ($p<0.001$; Fig.13). During spring, great tits were active 11.7 h, while blue tit and willow tit active for 10.2 and 10.7 hours respectively (Table 7). Longest activity time for all three songbirds were in the summer where great tits were active 16.4 +/ 1.7 h during polar day, and 3.7 h longer than willow tit ($p<0.001$; see Table 7 and Appendix D Table D-1 and D-2). Average temperature also had a statistically significant effect on activity time ($F=101.68$, $p<0.001$), they are active 10 minutes longer for 1 °C increase in temperature. The data analysis of activity time is on population level and will affect the overall activity time observed, and that comparison between the three species may be misleading due to difference in abundance of the three species.

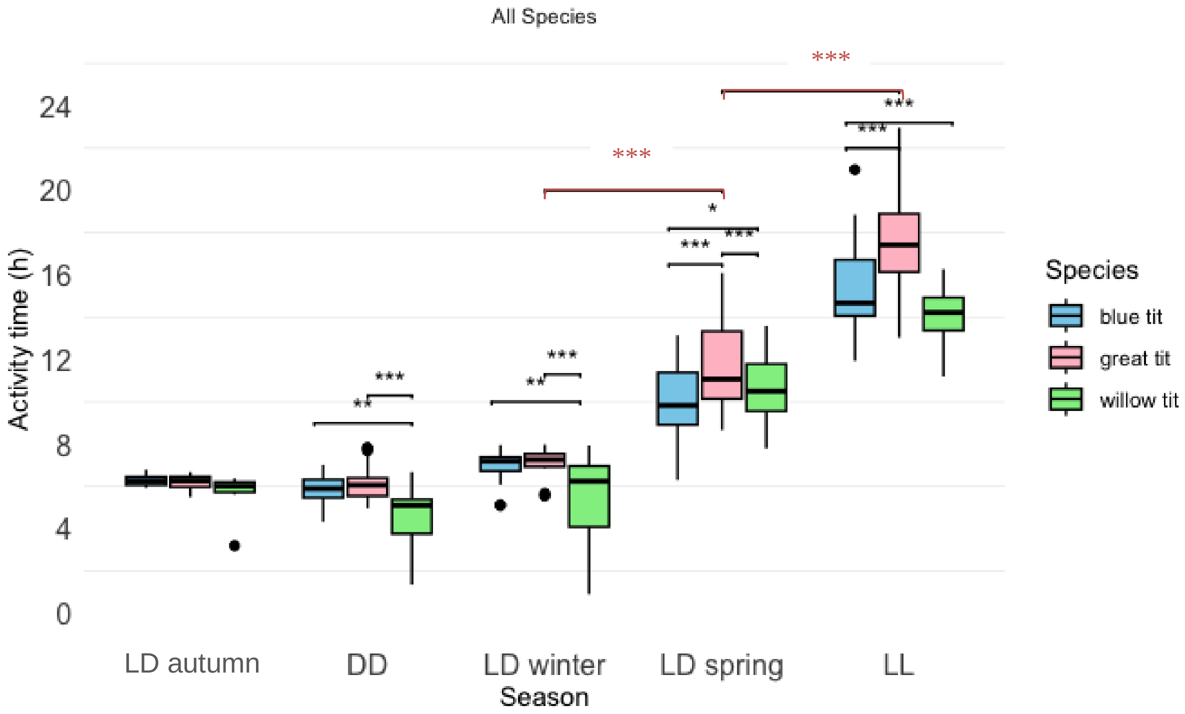


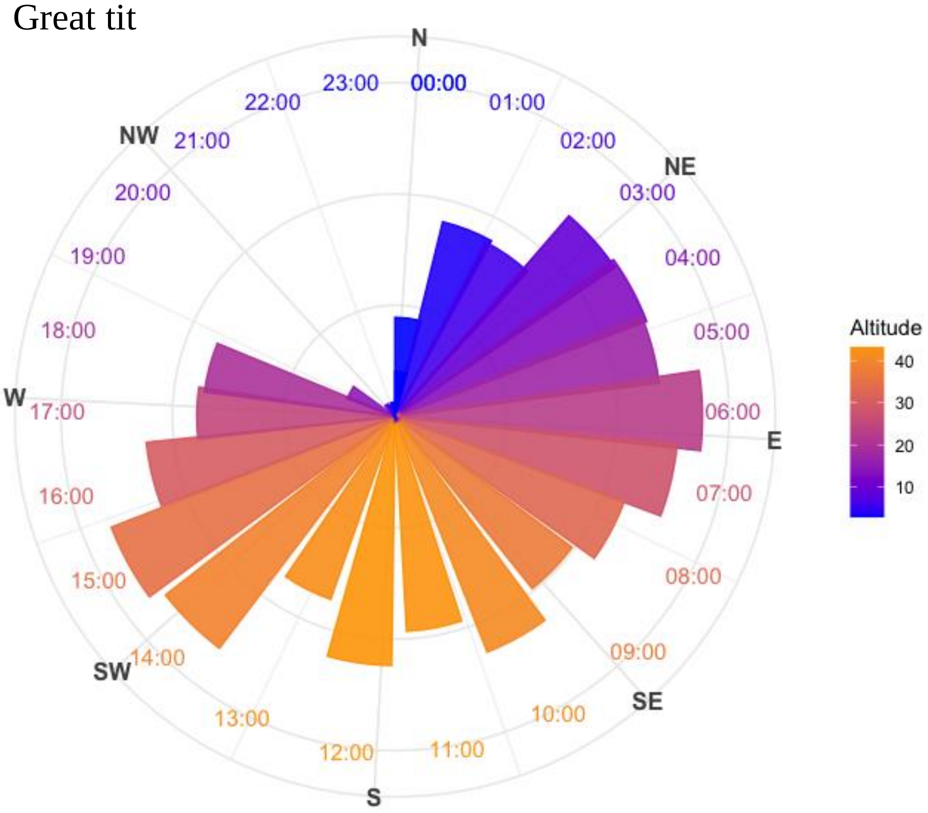
Figure 14: Duration of activity in hours at different seasons for blue tit (blue), great tit (pink) and willow tit (green). The horizontal lines illustrate statistically significant differences at threshold < 0.05 (*), 0.01 (**), and 0.001 (***) between the activity time between species at same season (black), and between closest season for the same species (orange), determined using the Tukey post hoc test. The black points indicate outliers.

Table 7: Interaction between season and species estimates of activity time and standard error in hours, and average temperature estimate and standard error of the multiple linear regression model with an adjusted R2 = 0.98.

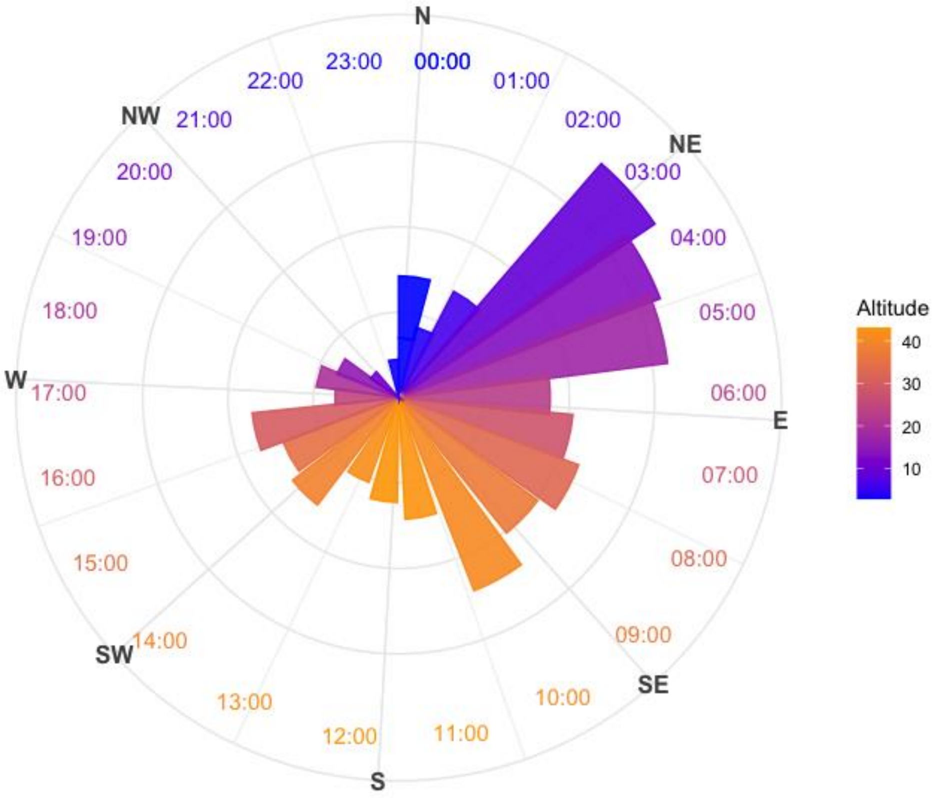
Season	**Blue tit**	**Great tit**	**Willow tit**
LD autumn	6.57 +/- 0.41	6.45 +/- 0.41	6.04 +/- 0.41
DD (polar twilight)	6.26 +/- 0.94	6.50 +/- 1.65	5.05 +/- 1.65
LD winter	6.81 +/- 0.98	7.12 +/- 1.73	5.17 +/- 1.75
LD spring	10.23 +/- 0.85	11.74 +/- 1.48	10.75 +/- 1.48
LL (polar day)	14.30 +/- 0.97	16.38 +/- 1.71	12.65 +/- 1.71
Avg. Temperature	0.17 +/- 0.02		

Polar plots of average number of observations at the feeders at population level per hour from polar days, June 1 to June 22. The plots illustrate the position of the sun, azimuth (north (N), east (E), south (S) and west (W)), and altitude is illustrated by colour, at the time of the day. Neither great, blue or willow tit takes advantage of the continuous daylight, instead, they rest in the evening, Great and blue tit are active more throughout the polar day than the willow tit (Fig. 15).

Great tit



Blue tit



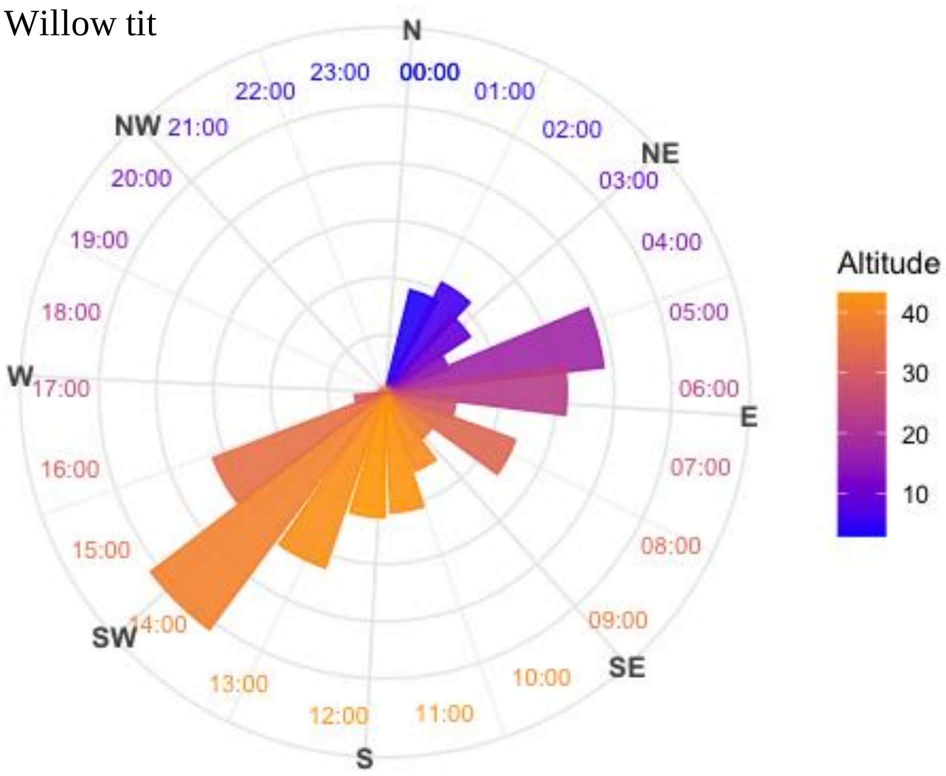


Figure 15: Polar plots average number of observations during polar summer (from June 1 to 22) for great, blue and willow tit.

3.2.1 Periodicity

Great, blue, and willow tits exhibit diel rhythmicity in their activity patterns, both in the presence of the natural light-dark cycle and during polar days. Lomb-Scargle periodogram analysis of a female great tit (A02) and a willow tit (WT_al_ye) around the spring equinox gave a period of approximately 23.9 h. During polar days, the diel activity rhythmicity persisted, with A02 displaying a period of approximately 23.7 hours, while WT_al_ye exhibited periodicity of 24 hours (Fig. 16).

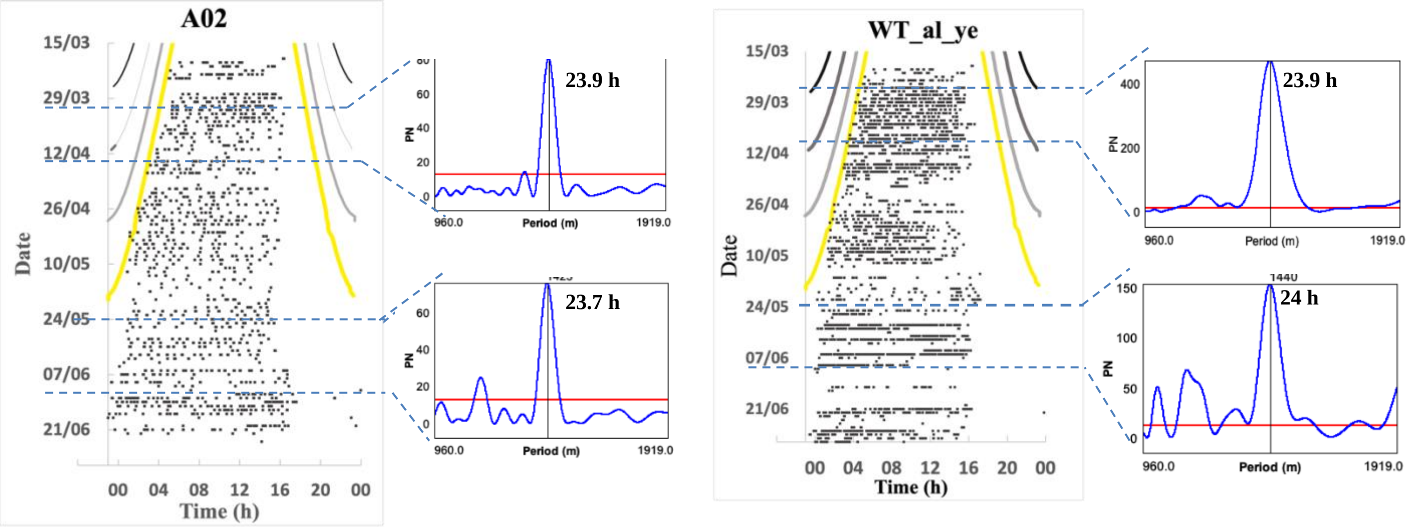


Figure 16: Individual activity data and periodicity A. Single plotted actogram illustrating female great tit individual A02 activity from 15. March to 30. June female. Date is illustrated on y axis and time of day (hour) on x axis. The grey lines indicate start and end of twilight phases: Astronomical twilight (dark grey line), nautical twilight (grey line), and civil twilight (light grey line), and sunrise or sunset (yellow line). **B.** Lomb-Scargle periodograms from LD spring and polar days (LL). The blue dotted lines indicates the period. Power normalized on y-axis and period in minutes on x-axis. Red line indicates significant level, α = 0.05.

3.3 Diel activity rhythms relative to the natural light-dark cycle

Figures 17 to 21 illustrate annual changes in phase angle differences (Ψ) between the phase marks of diel activity rhythm and phase marks of the natural light-dark cycle in hours for great tit (red), blue tit (blue) and willow tit (green).

3.3.1 The phase relationship between onset of activity and sunrise and civil dawn

Multiple linear regression of the Ψ-onset of both using sunrise and civil dawn as phase marks for the natural light-dark cycle show that both species, season and the interaction between species and season had an statistically significant effect on the phase relationship between the activity rhythm and the natural light-dark cycle (Sunrise: F = 3.18, p=0.014; civil dawn: F = 2.97, p = 0.007). The seasonal change of Ψ-onset for the three tit species are illustrated in Fig. 17 and 18A. Furthermore, the daily average temperature had also a statistically significant effect on the phase angle relationship (sunrise: F = 6.86, p= 0.009, civil dawn: F=20.20, p<0.001). Notably, the effect of temperature on the Ψ-onset is minimal, with a 2 minute later onset relative to civil dawn for each 1°C increase in temperature (Fig. 18B).

The time of onset of activity coincided with the time of sunrise the middle of March. Towards summer, the phase relationship is more stable (Fig. 17). Great tit start their activity in average 17 minutes after sunrise during LD spring, while willow tits and blue tits started activity 35 and 57 minutes after sunrise, respectively. However, the phase relationship between onset of activity and civil dawn is relative stable during autumn and the first week of polar twilight (Fig. 18A), where the tit species start their activity around 2-8 minutes after civil dawn.

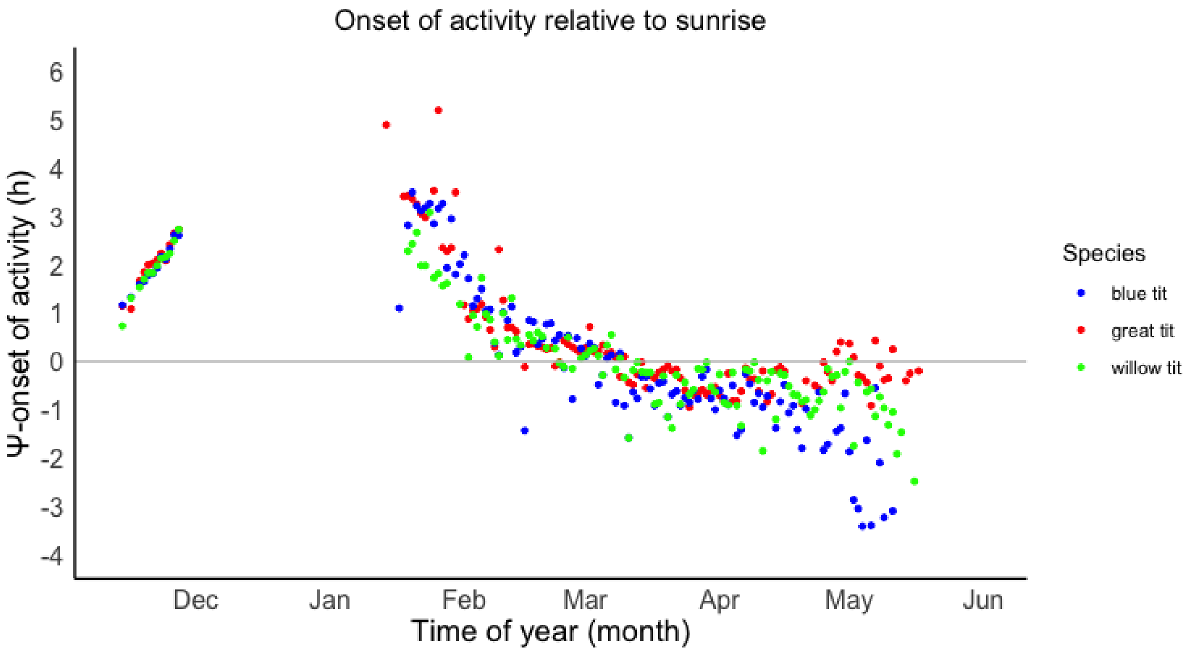


Figure 17: The seasonal change in onset of activity relative to sunrise (grey line) in hours. Species are represented with colour: blue tit (blue), great tit (red), and willow tit (green).

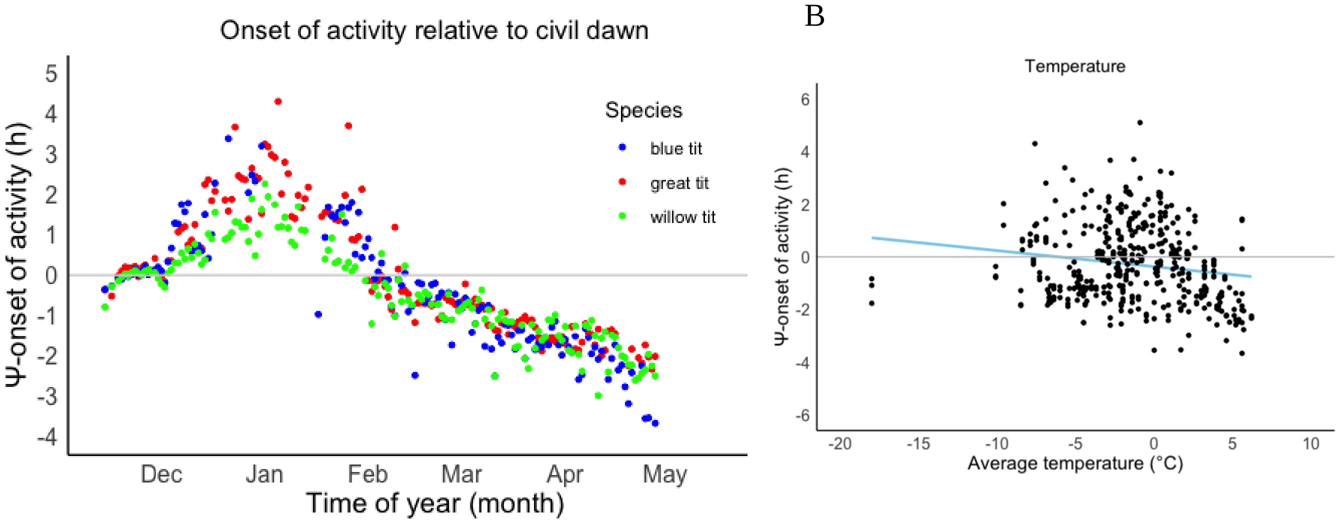


Figure 18:A. The seasonal change in phase-angle differences between the time of onset and civil dawn (grey line). Species are represented with colour: blue tit (blue), great tit (red), and willow tit (green). **B.** the effect of temperature. Blue line is the regression line from the fitted linear model.

The Tukey post hoc test revealed statistically significant differences of Ψ-onset between blue tit and great tit (mean difference: -0.74 h, p <0.001), and blue tit and willow tit (mean difference: -0.43 h, p = 0.04), during spring using sunrise as phase mark (Table 8). However, there were no statistically significant difference between the species during spring using civil dawn as phase mark. During polar twilight, there was a statistically significant difference between great and willow tit (mean difference: 0.89 h, p <0.001), and also between blue and great tit (mean difference: -0.59 h, p = 0.004; Table 9).

Table 8: Tukey Post Hoc - Interspecies differences in Ψ-onset (sunrise) within the season LD spring. Diff = species 2 – species 1, and standard error (SE) is in hours.

Species 1	Species 2	Diff	SE	t-ratio	p-value
Season: LD spring					
Blue tit	Great tit	-0.74	0.15	-5.061	<.0001
Blue tit	Willow tit	-0.43	0.14	-3.020	0.0054
Great tit	Willow tit	0.30	0.14	2.132	0.0337

Table 9: Tukey post hoc - Interspecies differences in Ψ-onset (civil dawn) during polar twilight (DD). Diff = Species 2 – Species 1. Diff and SE given in hours.

Species 1	Species 2	Diff	SE	t-ratio	p-value
Season: DD					
Blue tit	Great tit	-0.59	0.19	-3.072	0.0045
Blue tit	Willow tit	0.30	0.19	1.552	0.1214
Great tit	Willow tit	0.89	0.16	5.465	<0.001

The Tukey post hoc test revealed statistically significant differences in Ψ-onset between all season for each species except between LD autumn and LD winter (Table 10 and 11). There was only a statistically significant difference between LD autumn and LD winter for the willow tit population using sunrise as phase mark (p<0.01; Table 10).

Table 10: Tukey post hoc – Differences in Ψ-onset (sunrise) between seasons for each species. Diff = Season 2 – season 1. Diff and standard error (SE) are presented in hours.

Season 1	Season 2	Diff	SE	t-ratio	p-value
Blue tit					
LD autumn	LD winter	0.56	0.28	1.991	0.0472
LD autumn	LD spring	2.91	0.27	10.895	<.0001
LD spring	LD winter	-2.35	0.17	-14.044	<.0001
Great tit					
LD autumn	LD winter	0.48	0.28	1.701	0.0898
LD autumn	LD spring	2.24	0.27	8.429	<.0001
LD spring	LD winter	-1.77	0.17	-10.477	<.0001
Willow tit					
LD autumn	LD winter	0.86	0.29	3.012	0.0028
LD autumn	LD spring	2.44	0.27	9.191	<.0001
LD spring	LD winter	-1.58	0.18	-9.047	<.0001

Table 11: Tukey post hoc – Differences in Ψ-onset (civil dawn) between polar twilight (DD) and LD autumn, LD winter and LD spring for each species. Diff = season 2 – season 1, and SE are given in hours.

Season 1	Season 2	Diff	SE	t-ratio	p-value
Blue tit					
DD	LD autumn	1.14	0.27	4.258	0.0001
DD	LD spring	2.91	0.19	15.762	<.0001
DD	LD winter	0.97	0.19	4.989	<.0001
Great tit					
DD	LD autumn	1.65	0.25	6.713	<.0001
DD	LD spring	3.11	0.15	20.192	<.0001
DD	LD winter	1.44	0.17	8.611	<.0001
Willow tit					
DD	LD autumn	0.87	0.25	3.545	0.0009
DD	LD spring	2.38	0.15	15.662	<.0001
DD	LD winter	0.99	0.17	5.742	<.0001

3.3.2 The phase relationship between offset of activity and sunset and civil dusk

The seasonal change in Ψ-offset for great, blue and willow tits are illustrated in Figure 19 and 19A. Multiple linear regression analyses of the Ψ-offset using civil dusk as a phase mark, similar to the analyses of Ψ-onset, species, season and the interaction between species and season had an statistically significant effect on the phase relationship between offset of activity and the natural light-dark cycle (F = 3.34, p = 0.006). In contrast, using sunset as the phase mark for the natural light-dark cycle, the interaction between species and season was not statistically significant. However, both species and season individually showed statistical significance (species: F = 5.39, p = 0.005 and season: F = 258.96, p < 0.001). In addition, the daily average temperature had a statistically significant effect on the phase angle relationship (sunset: F = 101.32, p<0.001; civil dusk: F = 52.2, p<0.001). In average, the tits end their activity 6 – 7 minutes earlier relative to the sunset and civil dusk for each 1°C increase in temperature (Fig. 20B).

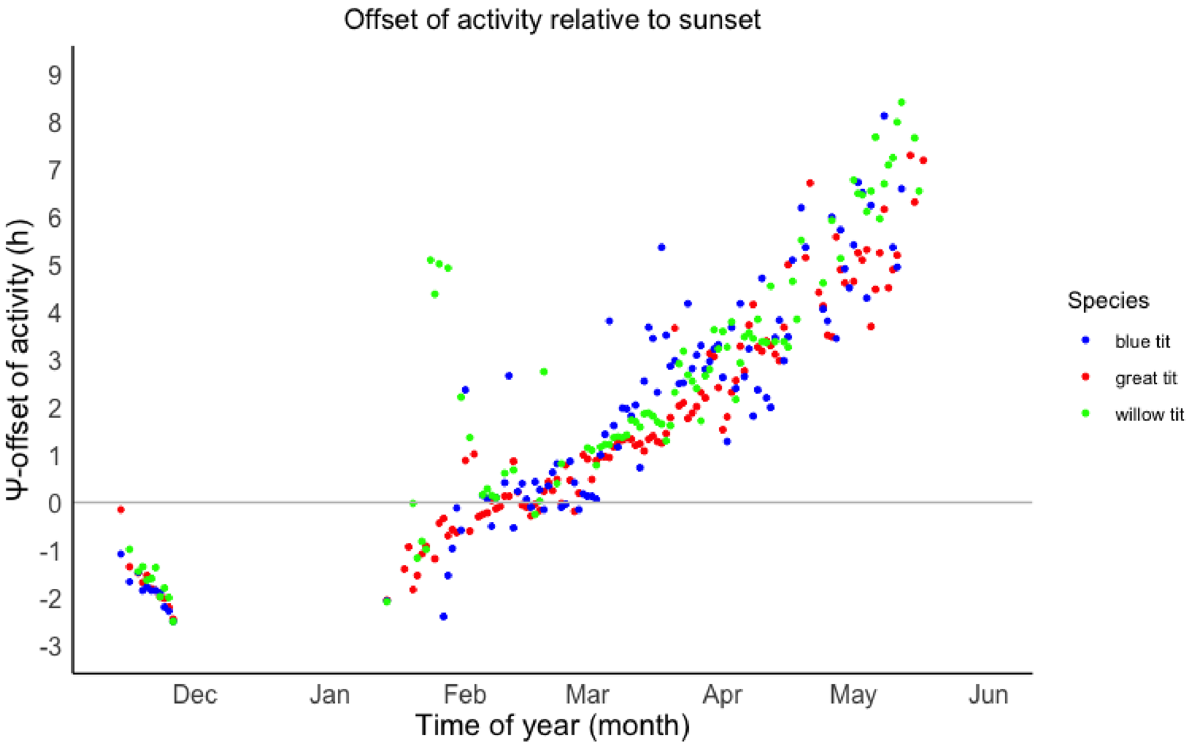


Figure 19: The seasonal change in offset of activity relative to sunrise (Ψ-offset) in hours. Ψ-offset = 0 (grey line) when offset of activity coincide with the time of sunset. Species are represented with colour: blue tit (blue), great tit (red), and willow tit (green).

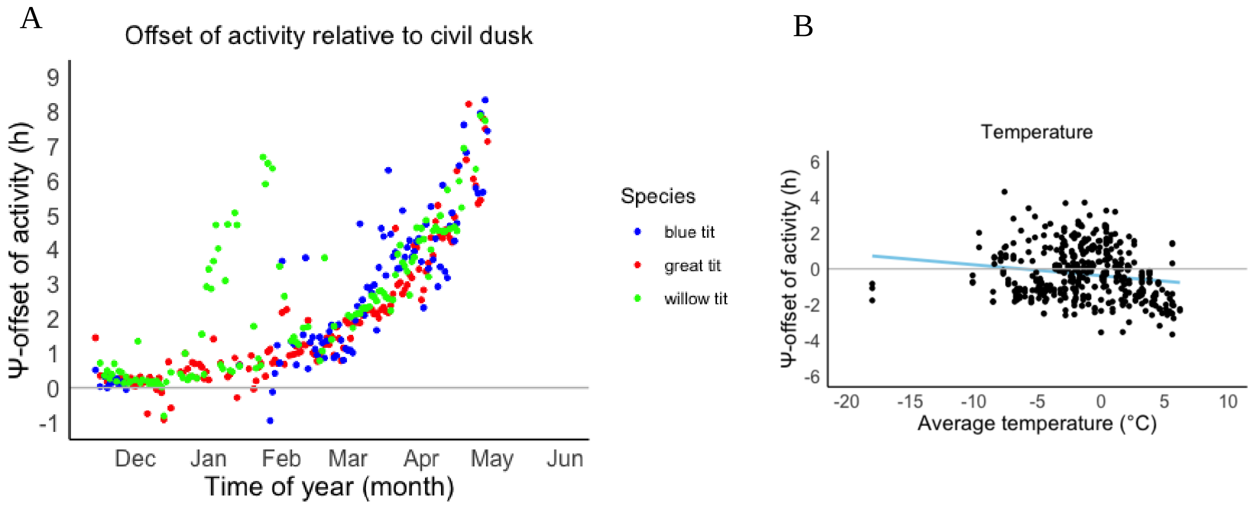


Figure 20: A. The seasonal change in phase-angle differences between the time of offset and civil dawn (Ψ-offset). Ψ-offset = 0 (grey line), when offset of activity coincides with the time of civil dawn. Species are represented with colour: blue tit (blue), great tit (red), and willow tit (green). **B.** the effect of temperature. Blue line is the regression line from the fitted linear model.

Pairwise comparison using Tukey post hoc test revealed only statistically significant difference in Ψ-offset (sunset) between great and willow tit (see Appendix D Table D-6). While using civil dusk as phase mark for the natural light-dark cycle there were a statistically significant difference between great tit and willow tit during polar twilight (p=0.02; Appendix D Table D-7), and between willow tit and blue and great tit during winter (p=0.02 and p = 0.003; Appendix D Table D-7). However, the offset of activity for willow tit varied a lot during polar twilight and the beginning of LD winter (Fig. 20A), which influence the results.

The phase relationship between offset of activity and the time of civil dusk is relative stable during autumn and during polar twilight. Great tits end their activity in average 33 minutes before civil dusk, while blue tits and willow tits end their activity 22 and 40 minutes before for civil dusk during autumn, respectively. There were no statistically significant difference between Ψ-offset during autumn and polar twilight (p=0.05; Table 12). The Ψ-offset increased toward summer, where they end their activity in average of 3.2 to 3.8 h before the time of sunset during spring. Right before polar days, the offset phase-lead sunset up to 8-9 hours (Fig. 19).

Table 12: Tukey post hoc - Differences in Ψ-offset (civil dawn) between seasons for each species. Diff = Season 2 – season 1, and SE is given in hours.

Season 1	Season 2	Diff	SE	t-ratio	p-value
Blue tit					
LD autumn	LD spring	-3.93	0.41	-9.671	<.0001
LD autumn	LD winter	-0.87	0.44	-1.999	0.0463
LD spring	LD winter	3.06	0.29	10.554	<.0001
Great tit					
DD	LD autumn	-0.02	0.43	-0.054	0.9568
DD	LD spring	-3.36	0.27	-12.313	<.0001
DD	LD winter	-0.57	0.29	-1.927	0.1643
LD autumn	LD spring	-3.34	0.41	-8.221	<.0001
LD autumn	LD winter	-0.54	0.42	-1.293	0.3941
LD spring	LD winter	2.80	0.26	10.625	<.0001
Willow tit					
DD	LD autumn	0.88	0.44	2.002	0.0460
DD	LD spring	-2.45	0.27	-8.979	<.0001
DD	LD winter	-0.89	0.34	-2.579	0.0206
LD autumn	LD spring	-3.33	0.42	-7.849	<.0001
LD autumn	LD winter	-1.77	0.47	-3.733	0.0007
LD spring	LD winter	1.56	0.33	4.785	<.0001

3.3.3 Midpoint of activity

There was no statistically significant interaction between species and season of the analysis of Ψ-midpoint. Both species and season had statistically significant effect on Ψ-midpoint (Civil dawn - civil dusk: species: F = 3.66, p = 0.03; season: F = 21.72, p <0.001). However, there were only a statistically significant difference between blue tit and willow tit (sunrise to sunset: mean difference: -0.35 h, p = 0.0019; See Appendix D Table D-8). The seasonal trend in Ψ-midpoint is overall the same for the three species (Fig. 21). The Tukey post hoc test revealed statistically significant differences in Ψ-midpoint between all season except between DD and LD spring (p = 0.10; Table 13).

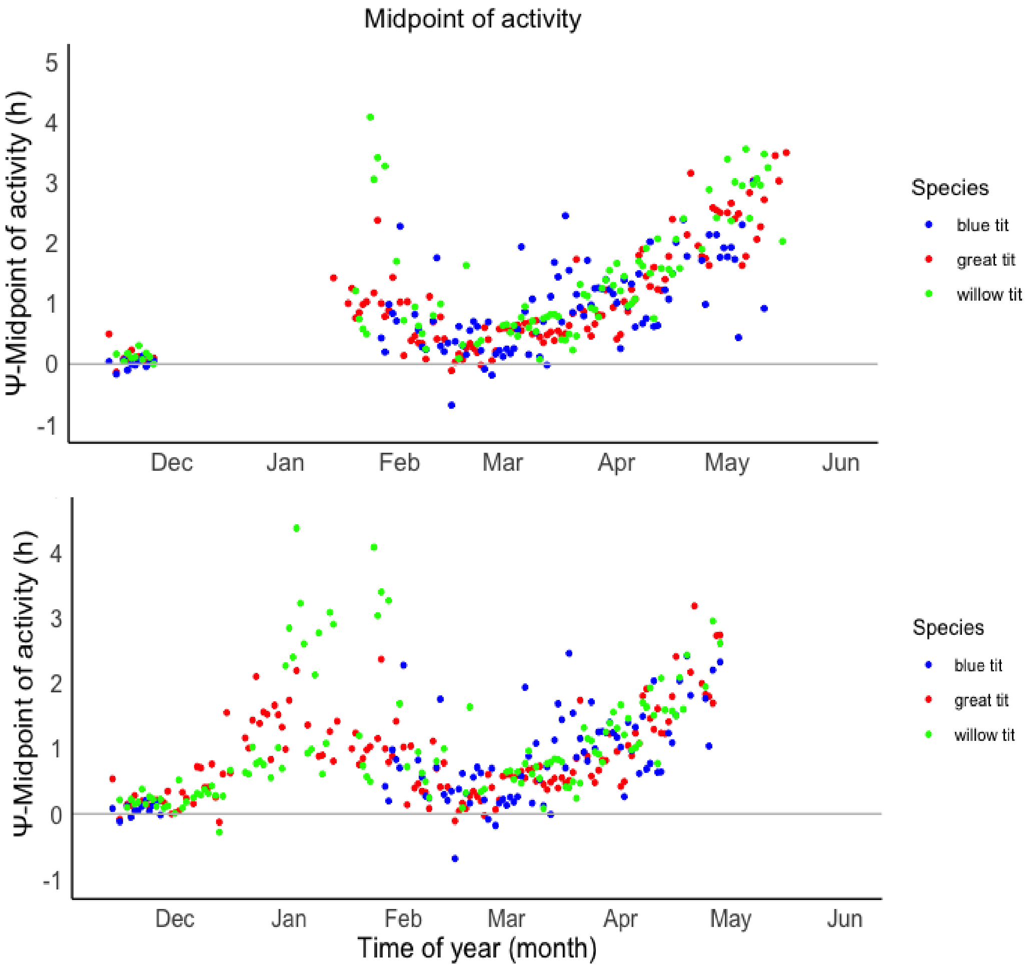


Figure 21: Phase angle difference between midpoint of activity and midpoint of the LD cycle. Top: sunrise to sunset, Bottom: civil dawn to dusk.

Table 13: Tukey post hoc - Differences in Ψ- Midpoint (from civil dawn to civil dusk) between seasons. Diff = Season 2 – season 1, and SE is given in hours

Season 1	**Season 2**	**Diff**	**SE**	**t-ratio**	**p-value**
DD	LD autumn	0.80	0.15	5.501	<.0001
DD	LD winter	0.27	0.12	2.354	0.0382
DD	LD spring	-0.17	0.10	-1.623	0.1055
LD autumn	LD winter	-0.53	0.14	-3.812	0.0005
LD autumn	LD spring	-0.97	0.13	-7.468	<.0001
LD spring	LD winter	0.44	0.09	4.706	<.0001

4 Discussion

The objectives of this thesis were to examine the differences in diel activity rhythms between three species of wild songbirds—the great tit, blue tit, and willow tit—in the subarctic, as well as the seasonal variations in diel activity rhythms. The study spanned over a time period that encompassed both polar twilight and polar day, including the rapid changes in photoperiod in between. This enabled the observation of the activity patterns of wild birds under various light conditions in a weak rhythmic environment using visit to feeders as a proxy for general activity, and the feeder visitation time as a proxy for the activity- rest rhythm.

4.1 Annual diel activity rhythms

The great, blue, and willow tit populations maintain diel activity rhythmicity throughout the seasons even in the absence of the natural light-dark cycle. Despite the reduction of the *Zeitgeber*, none of these bird populations become arrhythmic. This suggests that the observed diel activity rhythmicity is driven by the endogenous oscillator and its robustness. Notably, the annual diel activity rhythms differ among the three species of wild songbirds at 69°N. A distinct difference in the activity pattern is observed between the great and blue tit, which have more recently colonised northern latitudes, and the willow tit population, which has been inhabiting northern latitudes for a longer duration, when the natural light-dark cycle (no sunrise and sunset) is reduced (see Fig. 12).

The willow tit has a longstanding presence in the Arctic, whereas the great tit was observed breeding in Troms and Finnmark for the first time during the 1950s (Haftorn, 1957). The blue tit, less abundant than the great tit, increased its numbers in Northern Finland during the 1990s (Pakanen et al., 2018). Willow tits are better adapted to the cold temperatures in the north, in the terms of the foraging behaviour compared to great and blue tits (Pakanen et al., 2018). We can therefore rise the question if this the case for the Arctic light conditions as well.

Great and blue tits differ in their diel activity pattern from willow tits during polar twilight and polar day. This suggests distinct clock properties between the three populations which may reflect the time they colonised Northern areas. Nocturnal activity was observed during polar twilight for the great and blue tit populations, whereas this was not observed for willow tit. Haftorn (1994) observed diel foraging activity patterns of passerine birds, including great and willow tits, during polar twilight in Pasvik, Norway (69 °N). He did not report birds that exhibit nocturnal activity, which is in contrast with our observation. However, he only observed two feeders, located close to houses and streetlights, for two hours in the morning and the evening, and can therefore not exclude the possibility that the birds observed started their nocturnal activity during polar twilight. In addition, great tit started their activity in general earlier than willow and blue tit in our study. Great tit (excluding the nocturnal activity) started in average 48 min before willow tit during polar twilight, although the difference is not statistically significant, it aligns with other studies where great tit started to be active approximately 41 min earlier than willow tit in Pasvik (Haftorn, 1994).

Haftorn (1994) observed the birds with two feeders close to houses and artificial light at night (ALAN) was present. We also had weak background ALAN as the feeders were placed near the university along the ski trail illuminated by streetlight. The ecological and evolutional consequences of ALAN is in increasing interest as the amount of ALAN is rapidly increasing with urbanisation. ALAN have the potential to modify and disrupt the circadian system of wild animals and has shown to advance activity onset (de Jong et al., 2016), and some songbird species extend their activity duration during winter (Byrkjedal et al., 2012). McGlade et al., (2023) shown that the timing of onset of activity forest females that were exposed to ALAN were similar to urban females. There is also some evidence that great tits prefer to sleep under artificial light instead of darkness when they are given the choice (Ulgezen et al., 2019). ALAN may influence the tits differences in the timing of onset and the longer activity time due to their differences in roosting site. Great tits often roost closer to houses than willow tits, consequently exposed to more ALAN which may result in a masking effect.

Trends in onset of activity is that birds start activity earlier in the day in higher latitudes when compared to southern latitudes during winter. Haftorn (1994) compered the timing of diel foraging of passerine bird populations from Pasvik to populations in Klaebu (63 ºN), Southern Norway. He found that the bird population in Pasvik started and ended activity at lower light intensities than the southern counterparts. However, these observations do not tell us what exactly drives the observed difference in timing of onset of diel activity. Is it because of distinct clock properties which lead to a more sensitive inner time keeping system to light for northern bird populations, or is it a direct response to the colder winter conditions in the north, therefore, earlier onset of foraging to avoid starvation due to increased overnight energy expenditure? For example, other studies have shown that blue tits arrived later at feeders and great tits emerged later from their nest boxes when it was warmer (Fitzpatrick, 1997; Schlicht et al., 2023). We cannot exclude the effect of colder, harsher winter on the earlier onset of diel activity when comparing latitudes.

The coupling strength between the *Zeitgeber* and the endogenous oscillator depends on both the intensity of the *Zeitgeber* and the sensitivity of the endogenous oscillator to the *Zeitgeber* (Moore-Ede et al., 1982). During the polar twilight and polar day, the light intensity changes with lower amplitude. Consequently, the coupling strength between the diel activity rhythm and the change in light intensity is weaker for the three species during polar twilight and polar day. Given that the three songbird populations visit the same feeders and are exposed to approximately the same natural light conditions, it suggests that the differences in diel activity patterns may be attributed to variations in the sensitivity of the endogenous oscillator to light among the three species. The differences in circadian light sensitivity may involve circadian photoreceptors, downstream components like pathways between circadian photoreceptors and endogenous oscillators, or components of the endogenous oscillator. This raises the question of whether the inner timekeeping system of the willow tit population is more sensitive to light as an adaptation to the Arctic light conditions.

Latitudinal clines are commonly observed in biology and are typically considered adaptive evolutionary responses to latitudinal environmental gradients (Hut et al., 2013). Early predictions suggest a decrease in light sensitivity with latitude (Pittendrigh & Takamura, 1989). Activities during polar nights and polar days in arctic species support this, leading to arrhythmic behaviour. In contrast, Floessner et al. (2023) propose an increase in light sensitivity with latitude based on the northern line of *Nasonia vitripennis,* showing stronger phase resetting and a narrower distribution of the phase angle of entrainment over different durations of the light-dark cycle (T-cycles) compared to the southern line of *Nasonia vitripennis*.

The activity rhythm of great and blue tit populations observed during polar twilight is clearly not entrained by the daily fluctuations of light intensity during twilight cycles or other potential *Zeitgebers*, as it is too weak to entrain the activity rhythm. However, it appears that light still influences the phase of the activity rhythm, a pattern described as relative coordination. This phenomenon is frequently observed in various studies. For example, a bright-light/dim-light cycle did not entrain the wheel-running rhythms of hamsters; however, it did speed up the rhythm at certain phases and slowed it down at others (Swade and Pittendrigh, 1967). It is conceivable that when the time of the great or blue tit subjective dawn or dusk (when it is more sensitive to light) coincides with the time of the transition between nautical and civil twilight (when the light intensity changes are largest during polar twilight), it will influence the activity rhythm by speeding it up or slowing it down. Resulting in relative coordination where the *Zeitgeber* hits at different phases for each activity rhythm, where the period of the following activity rhythm depends on when in subjective day the phase hit. The relative coordination observed for great and blue tits during polar twilight could suggest disruption of the circadian system, and lead unfortunate timing of behaviour where they may be exposed to increased predation risk or it may have other health and fitness consequences.

During polar days, we observe a distinct difference in the activity pattern between great and blue tits from the willow tits, where great and blue tits started again activity before midnight when the natural light-dark cycle is reduced, whereas this was not observed for willow tits. In addition, great tit (A02) had a shorter periodicity than the willow tit (WT_al_ye) which had period of 24 h during polar day. However, more individual activity data has to be analysed to state if this is a general trend of great tits and willow tits in subarctic. Arrhythmicity was not observed for neither of the tits, and they show a clear diel activity rhythmicity. Both that the great and blue tits start their daily activity before midnight and the shorten periodicity may be in agreement with Aschoff´s rule which states that higher light intensity speed up the clock, thus shorten the period, in diurnal animals (Aschoff, 1960). Lapland longspurs, which is entrained during polar summer, held in light-controlled aviaries support the Aschoff´s rule, where the free-running period is shorter in birds held in constant light than in constant darkness (Ashley et al., 2014).

Neither of the tit species takes advantage of the daylength during polar days where they rest in the evening. The seasonal trend in daily activity duration is the same for the three tit species with an increase in activity duration with the longer photoperiod towards summer. The activity time changes less throughout the year than the solar day (sunset to sunrise), which similar to other studies (Daan and Aschoff, 1976). While the natural light-dark cycle becomes less pronounced in May 18th, great tit are active in average 16.5 hours, blue tit are active 14.4 hours, and willow tit 12.8 hours of the 24 solar day during polar summer. This consists with Arctic songbirds such as Lapland longspurs, which maintained distinct daily rhythm during polar summer (71 ^{o}N; Ashley et al., 2012; Steiger et al., 2013), as well as songbirds breeding at high arctic latitudes during the polar summer which rest around midnight (Karplus, 1952; Krüll, 1976). Great tit and blue tit use more of the day than willow tit (illustrated in Fig. 15).

The distinct difference in activity patterns, including the onset and duration of activity, between great tits and blue tits in comparison to willow tits during polar days, may also suggest differences in their circadian clock properties. The robust entrainment of willow tit activity rhythms during polar days may also suggest a heightened sensitivity to light cues which enable them to stay entrained although the natural light-dark cycle is reduced. During polar days, the tits are entrained by weaker *Zeitgebers*. It can be non-photic environmental cycles, e.g. cycles in ambient temperature, social cues or food availability (Moore-Ede et al., 1982). Or it can be other light cues than the light-dark cycle. Low amplitude light intensity cycles (Krüll, 1976a; Swade & Pittendrigh, 1967), and other periodic factors such as position of the sun, altitude, and azimuth, or change in spectral composition have been proposed as possible *Zeitgebers* (Krüll, 1976b, 1976c; Pohl, 1992, 1999).

In the high Arctic during polar day, there is a cycle of light intensities within a range exceeding 10 000 lux. Laboratory experiments have shown that many passerine birds tend to initiate continuous activity at high light intensities (Krüll, 1976a). Consequently, it is suggested that the low amplitude light intensity cycle have a little effect on entrainment during polar day. Additionally, experiments with arctic breeding passerine birds, such as brambling (*Fringilla montifringilla*) and the common redpoll (*Carduelis f. flammea*), demonstrated that these birds can entrain to cycles of spectral composition of light and relative position of an orbiting light source (Pohl, 1999; Krüll, 1976b). Further research is essential to deepen our understanding of how passerine birds entrain their diel activity rhythm during polar day and identify the specific environmental cycles that function as *Zeitgebers*.

Both field and experimental studies on animals living in the Arctic have shown high diversity in activity patterns. Difference in activity patterns in animals living in the Arctic may reflect difference in favourable strategies shaped by their physiological and ecological context. The Svalbard and Norwegian reindeer are large herbivores which can store large amount of fat. In addition, reindeers experience minimal predation pressure. Similarly, for the Svalbard ptarmigan. It may beneficial to build up fat storage to prepare for the cold winters when the food availability is scarce. On the contrary, small songbirds like tits face limitations in accumulating fat reserves, and an around-the-clock foraging activity may heighten the risk of predation. From previously studies there is no clear trend or a general rule for animals inhabiting the Arctic. It reasonable to think that an escape from the circadian dominance during polar night and polar day into a more flexible around-the-clock foraging activity is beneficial for reindeers and Svalbard ptarmigan. However, it seems likely that such around-the-clock foraging activity would not be beneficial for small songbirds living in the Arctic. This may reflect in that willow tit maintain a robust entrained activity rhythmicity when the natural light-dark cycle is reduced.

4.2 Diel activity rhythms relative to the diel solar light-dark cycle

The difference in phase angle (Ψ) changes with seasons and depends on the properties of the *Zeitgeber* and the endogenous oscillator. The properties of the endogenous oscillator that effects Ψ may be free-running period, amplitude and the sensitivity to the *Zeitgeber* which differ between species, population and individuals (Moore-Ede et al., 1982). This is an observational study of the diel activity pattern of wild songbirds in their natural environment, and therefore the properties of the endogenous oscillator are not measured. However, the properties of the *Zeitgeber* changed during the course of the study, which may explain the seasonal change in Ψ, and the differences between the species may reflect differences in properties of the endogenous oscillator.

We focused on the natural light-dark cycle, which is the most important *Zeitgeber* (Moore-Ede et al., 1982). The properties of the natural light-dark cycle, such as the ratio between light and dark, the strength, and the duration of twilight, change throughout the year as the earth orbit around the sun with its tilted axis. These theoretical considerations may influence the seasonal variation in Ψ between the activity rhythm and the natural light-dark cycle (Wever, 1967; Aschoff, 1969). The *Zeitgeber* is strongest around equinox, as we move towards winter or summer the light conditions move towards “DD” or “LL” conditions as the daylength gets shorter or longer, respectively. The *Zeitgeber* is weakest during the winter and polar twilight where there is no sunrise or sunset. The natural light-dark cycle is reduced during the polar summer, however, entrainment is still theoretically possible since the altitude of the sun exhibits diel variations (Schmal et al. 2020). As *Zeitgeber* strength increases, the Ψ becomes smaller, and it would be expected that Ψ coincide with the light-dark cycle around spring equinox when the light-dark ratio is approximately 1:1 between light phase and dark phase (Aschoff, 1969).

The determination of the phase relationship (Ψ) between the daily environmental cycle and the daily activity rhythm relies on the chosen phase marks and underlying assumptions. This includes considerations of whether a single oscillator (or a system of multiple oscillators, behaving collectively as one oscillator when synchronised), or a two-oscillator model that track dawn and dusk give rise to the diel activity periodicity (Aschoff & Wever, 1962; Aschoff, 1969). In addition, onset of activity are more closely linked to sunrise than offset of activity are to sunset throughout the year (Daan and Aschoff, 1976). Great tits exposed to different artificial light conditions had a greater effect on onset, whereas the effect on offset was more limited (de Jong et al., 2016). This suggest that changes in light intensity is a stronger cue for timing onset than for timing of daily activity. However, if the diel activity rhythmicity is not based on a two-oscillator model, it makes less sense to use onset and offset as phase marks. Therefore, the midpoint of activity time may serve as a better phase mark for the activity rhythm (Aschoff & Wever, 1962; Aschoff, 1969).

4.2.1 Phase relationship between onset of activity and the natural light-dark cycle

When we compared the activity onset of the birds relative to the sunrise, we only found statistically significant differences in Ψ between blue tit and great tit during spring ($p<0.001$). In contrast, there was no statistically significant difference between great and blue tit during spring using civil dawn as phase mark. Right before polar days, the first registered visit at the feeders varied more for blue tits. This is probably due to less blue tits visiting the feeders and several day with overall few visits from blue tits during late spring and summer which may be due to increased natural food availability and reproduction state may play a role. During polar twilight, we only found statistically significant differences in Ψ-onset using civil dawn as phase mark between great and willow tits. Great tits started their activity 97 min before civil dawn, while willow tits started their activity in average 44 min before civil dawn during polar twilight.

The Ψ-onset changed throughout the year and the three species show the same seasonal pattern in ψ-onset (excluding the nocturnal activity) as illustrated in Figure 17 and 18A. When we compared the Ψ-onset between season we found statistically significant differences between autumn and spring, and winter and spring for the three species. Based on previously studies, the seasonal trend in ψ-onset is that the largest ψ-onset is in the midwinter and smallest ψ-onset in midsummer. However, most studies are from location below the Arctic circle (Daan and Aschoff, 1976). Our results are similar, with the largest ψ-onset right before and after polar twilight in the winter, and the smallest ψ-onset late spring except for great tit, and that onset coincide with sunrise in the beginning of March (Fig. 17). Our findings support the generalization that the onset of activity starts earlier in the day but later relative to sunrise with an increasing photoperiod. These observations are consistent with both field and captive animals in a natural light-dark cycle (Aschoff, 1969; Daan and Aschoff, 1976). In the autumn and first week of polar twilight the onset coincided closely with civil dawn. On average, the tit populations began their activity 2 to 8 minutes after civil dawn in autumn. The onset of activity phase-led civil dawn during polar twilight, coincided again with civil dawn in early February, and phase-lagged civil dawn in spring. During spring, blue tits initiated their activity, on average, 1.9 hours after civil dawn, while great tits and willow tits started, on average, 1.5 and 1.6 hours after civil dawn, respectively. The seasonal pattern of Ψ between the onset of activity and civil dawn for our tit populations at 69° N resembles that of the Svalbard ptarmigan caged at 79° N, in contrast to the Svalbard ptarmigan caged at 70° N, which had a more stable phase relationship between onset and civil dawn (Reierth & Stokkan, 1998).

4.2.2 Phase relationship between offset of activity and the natural light-dark cycle

When we compared the activity offset of the birds relative to the sunset, the Ψ between offset and sunrise exhibited significant differences only between great tits and willow tits ($p = 0.003$). Similarly, the Ψ between offset and civil dusk differed solely among species during polar twilight and winter. However, this is most likely due to fewer willow tits visiting the feeders and the increased variability in the timing of last visit during winter for willow tits. Thus, may not accurately reflect their overall activity rhythm.

The three tit species showed the same trend in the seasonal change in Ψ-offset. There were statistically significant differences between season for Ψ between offset and sunset, and there were also a statistically significant seasonal change Ψ-offset for all three tit species using civil dusk as phase mark for the natural light-dark cycle except between autumn and polar twilight, and polar twilight and winter. In contrast to the Ψ-onset, at higher latitudes, the trend is that the smallest Ψ-offset is in midwinter and the largest Ψ-offset in midsummer (Daan and Aschoff, 1976). Our results are similar, with the smallest ψ-offset, using sunset as phase mark, right before and after polar twilight and largest right before polar day. The seasonal change of ψ-offset of the three tit populations at 69° N are similar to what Daan and Aschoff (1976) observed for birds captive at the Arctic circle. The offset of activity relative to civil dusk for the three species follow the same trend, except during polar twilight. During autumn and first week of polar twilight the phase angle relationship is relative stable. They ended their activity in average 20 – 40 minutes before civil dusk in the autumn. Thereafter, in the spring they phase-led civil dusk in average 3.9 to 4.3 h and increases their activity up towards 9 hours before civil dusk in the end of the spring. The seasonal change in Ψ-offset are similar to what observed for Svalbard ptarmigan at 70 and 79 °N (Reierth & Stokkan, 1998).

4.2.3 Summary: seasonal change in onset and offset of daily activity

Aschoff & Wever (1962) formulated a general rule, stating that the onset of daily activity occurs at lower light intensities than the offset of activity (may be reverse during winter), based on field observations of vocalization and roosting times of birds. Observations of diel activity rhythms of captive birds by Daan and Aschoff (1976) and Reierth & Stokkan (1998) have supported this pattern, and it appears to hold true for our wild songbirds in subarctic. The onset of daily activity is more precise and follow the sunrise/ civil dawn more closely than offset of activity, reflected by the seasonal change in Ψ-onset are smaller than the Ψ-offset, which consistent with findings from other studies (Daan and Aschoff, 1976; Reierth & Stokkan, 1998). However, in contrast to Svalbard ptarmigan and songbirds held captive at the Arctic circle (Reierth & Stokkan, 1998; Aschoff, 1969), the wild songbird populations at Tromsø had a larger seasonal change in Ψ-offset. In addition, we observed that the wild songbirds ended their activity at approximately the same clock time starting from the beginning of March, as illustrated in Figure 13 (see also Table 3). Our observation does not match the two-oscillator model proposed by Pittendrigh (1974) as onset of daily activity seem to “track” dawn, however, end of daily activity do not “track” dusk throughout a large portion of the study period as the offset of activity do not change with the change in the time of civil twilight. The diel activity rhythm of great, blue and willow tit can therefore be better explained by a one-oscillator model. It could be possible that they just stopped visiting the feeders at the approximately same time each day. However, offset of other behavioural activities such as incubation and nest box activity was at approximately the same time as the last observed visit at the feeders (see Appendix F, Fig. F-2.3) Although, we cannot exclude the possibility for a two-oscillator model.

4.2.4 Midpoint of activity

When we compared the Ψ – midpoint of the birds, we only found a statistically significant difference in Ψ – midpoint (sunrise to sunset) between blue and willow tits. In contrast, there were no statistically significant difference in Ψ – midpoint between the birds when using civil dawn to dusk. Therefore, the difference reflects most likely the increased variation the time of the last registered visit at feeder during winter for willow tits, and the variation of the time of the first registered visit for blue tits during late spring.

The seasonal change in Ψ – midpoint is mostly reflected by the seasonal change in Ψ – offset, there were statistically significant differences in Ψ – midpoint between all seasons, except between polar twilight and spring. During autumn, the midpoint of activity phase-led on average 5 – 30 min the midpoint of the solar day. The phase relationship increased towards polar summer. This is consistent with the "seasonal rule" formulated by Aschoff (1964), which suggests that the increase in photoperiod should result in an increase in phase-angle differences in diurnal animals. In addition, the seasonal pattern of Ψ-midpoint for the three tit species aligns with the predictions that Ψ-midpoint in diurnal birds is maximized in summer, minimized around the equinox, and increases again towards winter (Aschoff, 1969). As illustrated in Figure 21, Ψ-midpoint are largest right before the reduction in the natural light-dark cycle in spring, and minimized around March, and increased differences in the phase relationship when the sun reappears again after polar twilight (sunrise to sunset), and have “peak” in the middle of polar twilight (civil dawn to civil dusk). Seasonal pattern of Ψ -midpoint of our wild songbirds in the subarctic are similar to that of captive Svalbard ptarmigan (Reierth & Stokkan, 1998). The magnitude of the seasonal change in Ψ-midpoint increases with latitude. At the Arctic circle, for species like the great tit and yellowhammer (*Emberiza citrinella*), the Ψ-midpoint was approximately 2 hours, whereas at lower latitudes it approximately 10 minutes (Aschoff, 1969). The Ψ-midpoint towards summer was up to 4 hours for the wild population of great, blue and willow tits at 69°N.

4.3 Other factors may influence the observed diel activity

This is an observational study of wild songbirds in their natural environment which means that we cannot exclude that other factor than the daily fluctuations in light intensity influenced the observed daily activity. For instance, a study by Lehmann et al. (2012) demonstrated that great tits subjected to artificial light-dark cycles at 18°C exhibited later onset and cessation of activity compared to those at 8°C. The visits to feeders are also linked to birds' foraging behaviour, balancing the risks of predation and starvation. During colder days, increased energy demands may lead to an earlier onset of activity to avoid starvation (Bonter et al., 2013). Additionally, rainfall may influence the timing of diel activity where heavy rainfall causes later onset and earlier offset (Kluijver, 1950; Hinde, 1952; Bruni et al. 2014, Da Silva et al. 2016; Schlicht & Kempenaers, 2020). We found that average temperature had a statistically significant effect on the parameters of the activity pattern analysed such as onset and offset. However, the effect was minimal and temperature is linked with season as it become warmer towards summer. Therefore, we anticipate that temperature and rainfall do not substantially alter the overall diel activity pattern, as the natural light-dark cycle remains the primary entraining factor.

Social interaction and dominance, both within the studied songbird species and with other passerine birds not included in the study, likely impact the number of observed feeder visits. Dominance often correlates with body size, with larger species typically dominating smaller ones. In spring, finches, being larger, dominated the feeders. Great tit, generally dominate blue tit (Hansen, 2004). Aggressive behaviour of great tit, and other bird species such as the greenfinch, was observed. We assume that the increased visits of finches during the spring have an effect of the number of observed visits at the feeders of the three tit species. However, how much it affected timing of activity is unknown.

Given the population-level analysis, individual distinctions are not possible. Yet, factors like sex and age may influence behavioural timing during different seasons, particularly noticeable during the breeding season (Steiger et al., 2013). Other studies show that male great tits show earlier onset than females in spring, with no difference observed in winter (Kluijver, 1950). Furthermore, the age of male blue tits affects activity timing, where adult males emerged and start to sing earlier then yearling males both during the non- and breeding season (Schlicht & Kempenaers, 2020; Steinmeyer et al. 2010; Poesel et al,. 2004).

Visit at feeders is used as a proxy for diel activity rhythm. During summer, the three species of songbirds visited the feeders less frequently. While there could be several explanations for this observation, we cannot definitively pinpoint the reason, and it is likely a combination of various factors. The reduced feeder visits might be attributed to an increased abundance of natural food sources and/or the availability of more favorable food options. In autumn and winter, these birds primarily rely on seeds and are commonly seen at feeders. Although our feeders were stocked with sunflower seeds, songbirds generally prefer insects. Their diet shift as the availability of insects increases towards the summer (Cowie & Hinsley, 1988).

In Tromsø, the great tit and blue tit enter their reproductive period in late spring. We observed minimal nest materials in some of the nest boxes at the end of April, and the first egg was laid on the 9th of May. During this time, they may invest more time in behaviours beyond foraging, such as nest-building, laying eggs, incubating, and the male providing food to the female at the nesting site. Therefore, other behaviours may serve as a better proxy for activity during this period than visits at feeders.

4.4 Camera-trap to measure diel activity pattern of wild songbirds

Camera traps have been used to collect the annual diel activity pattern of wild songbirds at lower latitude which give valuable activity data over seasons (Schlindwein et al., 2023). The utilization of camera traps provided extensive and valuable activity data for the three species of wild songbirds in subarctic in our study. Employing camera traps offers a non-intrusive method to monitor animal activity, ensuring minimal disturbance to wild species while delivering accurate data. In addition, camera traps are easy to handle.

However, there are few disadvantages with this method which make the data processing timing consuming and effect the activity dataset. The camera traps collect only activity displayed in front of them. It gets triggered by a slight movement in front of the camera which led to thousands of pictures of nothing. As the weather changes with wind, snow and rain lead to movement of the feeder, leaf or branches which triggered the cameras (see Appendix F). It is also very time consuming to go through the many thousands of pictures to detect if there is some with one of our species of interest among all the pictures of nothing. Moreover, the camera trap captures not only the target bird species but also all other bird species visiting the feeder. During the spring and approaching summer, the diversity and the number of birds visiting the feeders increased, leading to an abundance of pictures featuring other passerine birds beyond the great, blue, and willow tits in focus. Consequently, this also led instance where the feeders became empty during the study which may lead to periods where less birds visit the feeders during and right after the feeders were empty for sunflower seeds. Additionally, cold weather during winter led to the batteries were exhausted quickly, resulting in temporary camera failures. During these periods, no visual data were recorded, leading to periods where activity data were only recorded from one or two feeders. Unforeseen technical issues led to data loss. These issues were caused by memory card and storage drive corruption, they resulted in the inability to retrieve footage from specific time periods. There were two instances of data loss: from April 18th to April 23rd for feeder 2 and from May 18th to May 23rd for feeder 3.

The camera-trap and feeder set-up change during the study. Feeders 1 and 2 were replaced with trap box. The purpose of the change was to be able to capture birds to ring them to collect individual activity data. However, this change may affect bird activity. It was observed that certain birds, exemplified by a willow tit (ringed with a blue color ring, WT_al_lb), appeared hesitant or scared to enter the trap box for some months. WT_al_lb was only observed hanging upside down under the trap box, until the end of June where WT_al_lb was observed inside the trap box for the first time (see Appendix E). This behavioral reaction may have implications for the collected activity data.

Our method for collecting individual activity data was challenging, as it relied on the visual identification of rings on the birds feet. It was easier to identify individuals when we change the triggering interval from 5 s to 1 s. However, this was not sustainable for a longer period. Artificial intelligence (AI) is an increasing tool used in many aspects, which may be valuable tool to reduce the hours spend going through every image as it has the potential to filtering out the images without birds. Furthermore, automatic species recognition would reduce the time spent on data processing. Other methods may better way to collect individual activity, such as radio-telemetry using radio-frequency identification (RFID).

4.5 Notes on improvements of field work

Monitoring activity of wild songbirds started up in the winter 2022 in part of a longer project. The first camera was placed out in November. Weather is specially challenges during winter in Tromsø where most days consist of snow, wind and rain. I would have improved the setup by constructing a more protective configuration for the camera to prevent issues such as snow covering the lens or condensation, as well as make the feeder more stable right away. This would have reduced the days with poor quality images where it was impossible to distinguish the birds and reduce the amount of images of nothing. As well, modified the feeder in such way that it suited better for the tits, but not finches as they differ slightly in feeding preference. Tits are commonly observed hanging on to feeders, which is in contrast with finches. Therefore, removal of the bottom of the feeders may have reduced the amount of finches visiting the feeders. Six feeders were placed, I would have probably reduced and focused on 4 feeders instead as I was not able to go through everything, and placed them further apart to cover a larger area and more birds as the same birds visit the feeders 1, 2 and

3, as well as the same birds visit feeder 5 and 6. Placing nest boxes started also winter 2022, before the snow covered the ground. I would have placed them higher up (2 meters above the ground), as we needed to adjust them as they became almost covered by snow.

5 Conclusion

The seasonal light regime in the arctic influence clock output, here visits at the feeders as proxy for activity, differently in great and blue tit compared to willow tit. The three wild songbird populations show diel activity rhythmicity throughout the year. Great tits and blue tits have a distinct difference in their diel activity pattern compared to willow tits during polar twilight and polar days when natural light-dark cycle is reduced. Willow tits maintain a robust entrained rhythmicity throughout year, whereas great tit and blue tit show a pattern of relative coordination during polar twilight. This suggest that willow tits is more sensitive to the low-amplitude daily cycle of light intensity that they experience. In contrast, the low-amplitude daily cycle of light intensity is too weak to entrain the activity rhythmicity of great and blue tits during polar twilight. The relative coordination observed for great and blue tit could suggest times of circadian disruption. The effects this has on other aspects of physiology and fitness is unknown. Hence, further studies should focus on identifying this.

6 Works cited

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Appendix

Appendix A: Location of nest boxes and feeders

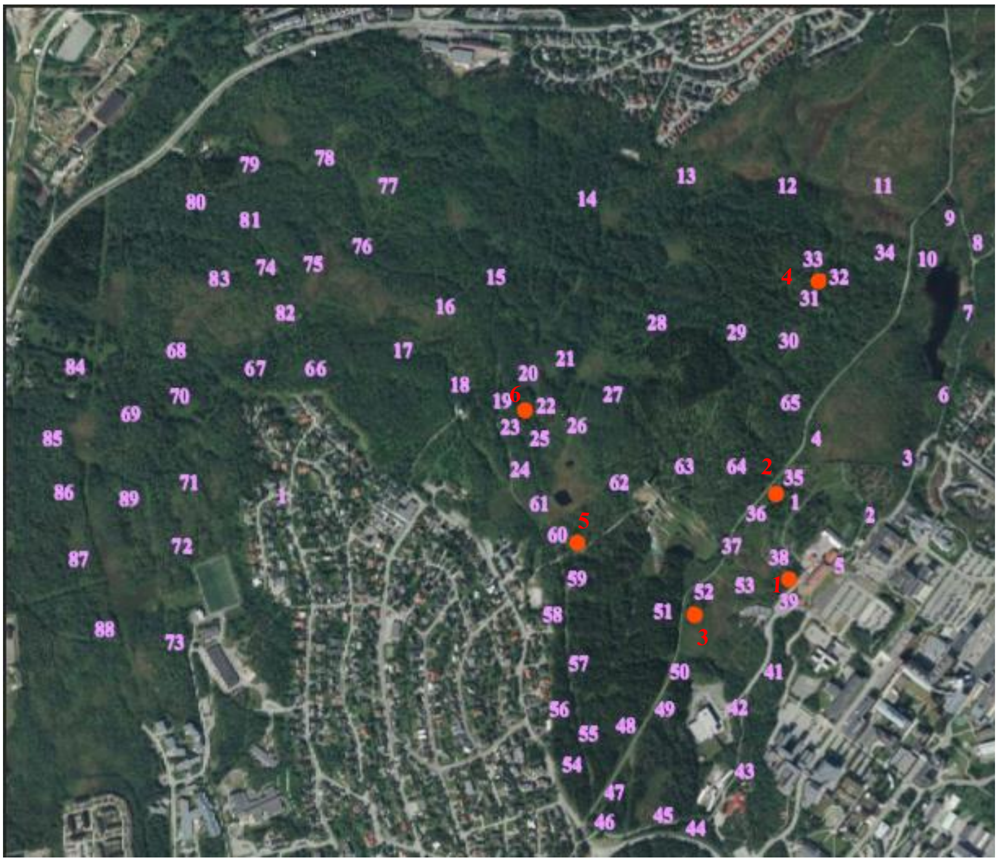


Figure A -1: Complete study site. Purple numbers represent nest boxes and the orange points present feeders.

Appendix B: Individual data

Table B-1: List of individuals which were ringed during the study period. Species and sex, and ID and colour ring, and when they were ringed.

SpeedyMouse-Code	Location	Ring	ID	Species	Sex	Left_Top	Right_Top	Right_Bottom	RingDate	RingTime
A01	Feeder 1	EM84001	A01	great tit	male	blackID	light blue	alu	19.mar	10:50
A51	Feeder 2	EM84004	A51	great tit	female	blackID	red	alu	19.mar	13:30
BT_al_ye	Feeder 3	HN34001		blue tit	male	alu	yellow		19.mar	15:00
A53	Feeder 4	EM84005	A53	great tit	male	blackID	pink	alu	19.mar	15:10
WT_lb_al	Feeder 5	HN34002		willow tit		light blue	alu		19.mar	15:30
WT_ye_al	Feeder 6	HN34003		willow tit	female	yellow	alu		19.mar	15:30
A02	Feeder 7	EM84006	A02	great tit	female	blackID	green	alu	27.mar	15:38
A03	Feeder 8	EM84007	A03	great tit	male	blackID	yellow	alu	27.mar	15:43
A52	Feeder 9	EM84008	A52	great tit	male	blackID	alu	red	27.mar	15:48
WT_re_al	Feeder 6	HN34004		willow tit	female	red	alu		06.mai	08:30
WT_gr_al	Feeder 6	HN34005		willow tit		green	alu		06.mai	08:30
A04	Feeder 6	EM84017	A04	great tit	female	blackID	alu	light blue	06.mai	09:56
A55	Feeder 6	EM84018	A55	great tit	male	blackID	alu	yellow	06.mai	10:02
A54	Feeder 2	EM84021	A54	great tit	female	blackID	alu	pink	06.mai	14:21
WT_pi_al	Feeder 4	HN34006		willow tit	female	pink	alu		16.mai	10:06
BT_al_re	Feeder 2	HN34008		blue tit	female	alu	red		24.mai	09:00

Scoring individuals in Speedymouse:

Example of scoring a great tit:

Species: parus_major
Individual: A01*
Sex: parus_major_m**

*In case the of an unringed individual, either unringed_m or unringed_f was applied.

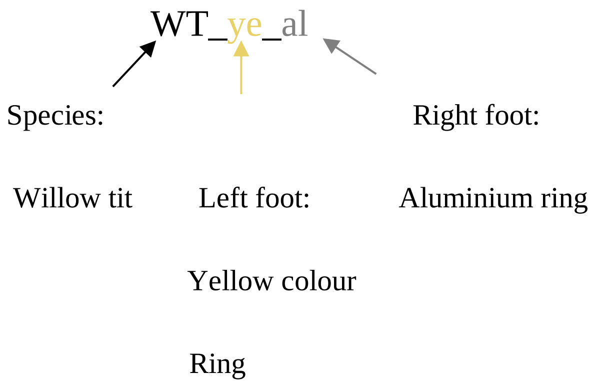
**m stands for male, parus_major_f in case of a female.

Example of scoring a willow tit (same way for blue tit):

Species: poecile_montanus

Individual: WT_ye_al

What the position and codes means:



Appendix C: Supplementary figures

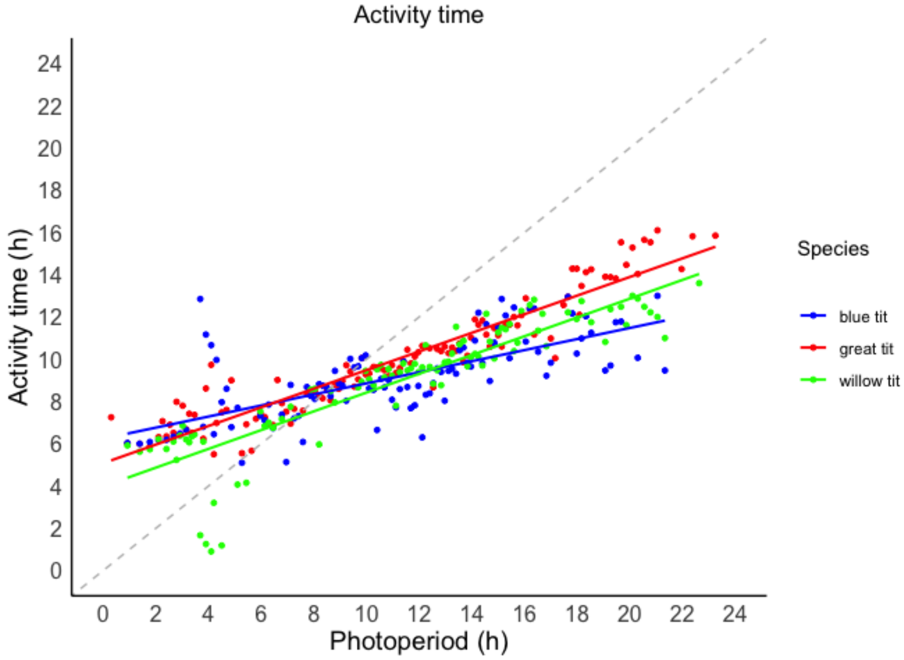


Figure C - 1: Activity time relative to photoperiod (sunrise to sunset), with different colour points for each species. The coloured lines are the regression lines from fitted linear model with an adjusted $R^2 = 0.98$.

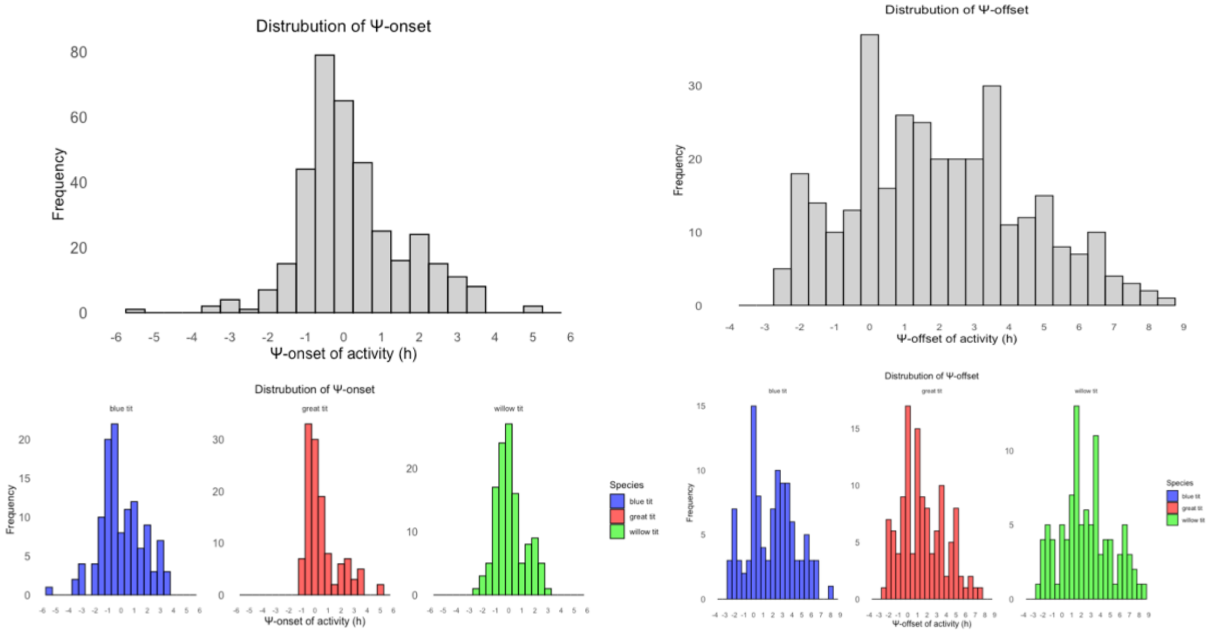


Figure B – 2: Distribution of Ψ-onset (A and B) and Ψ-offset (C and D). A and C represent the Ψ-onset and Ψ-offset for the three species combined. B and D represent the Ψ-onset and Ψ-offset for blue tit (blue), great tit (red) and willow tit (green).

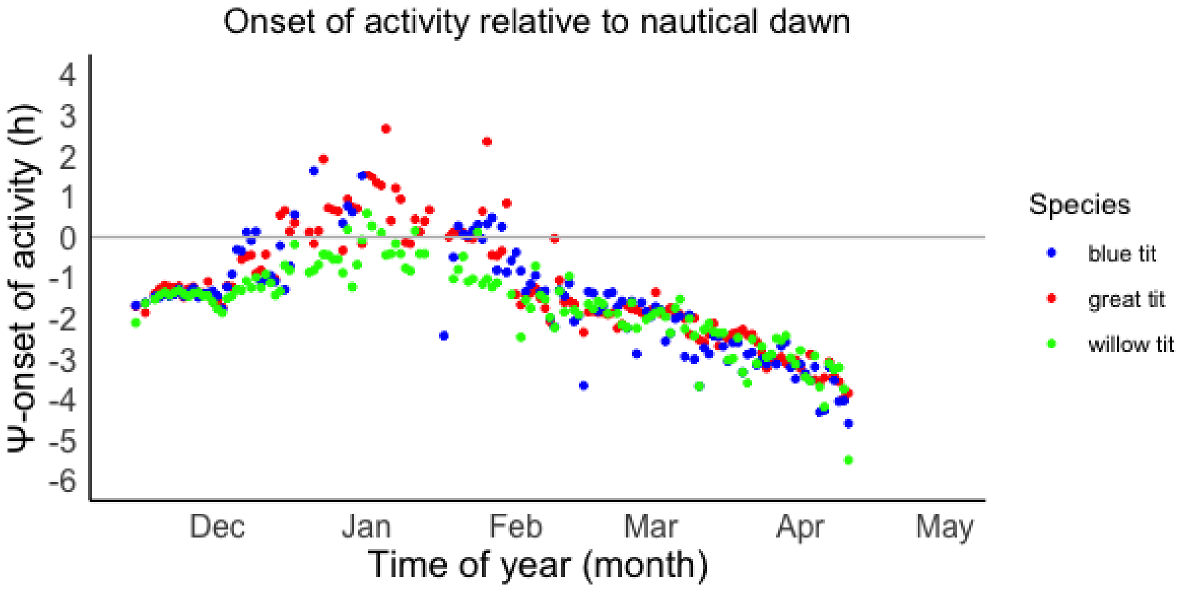
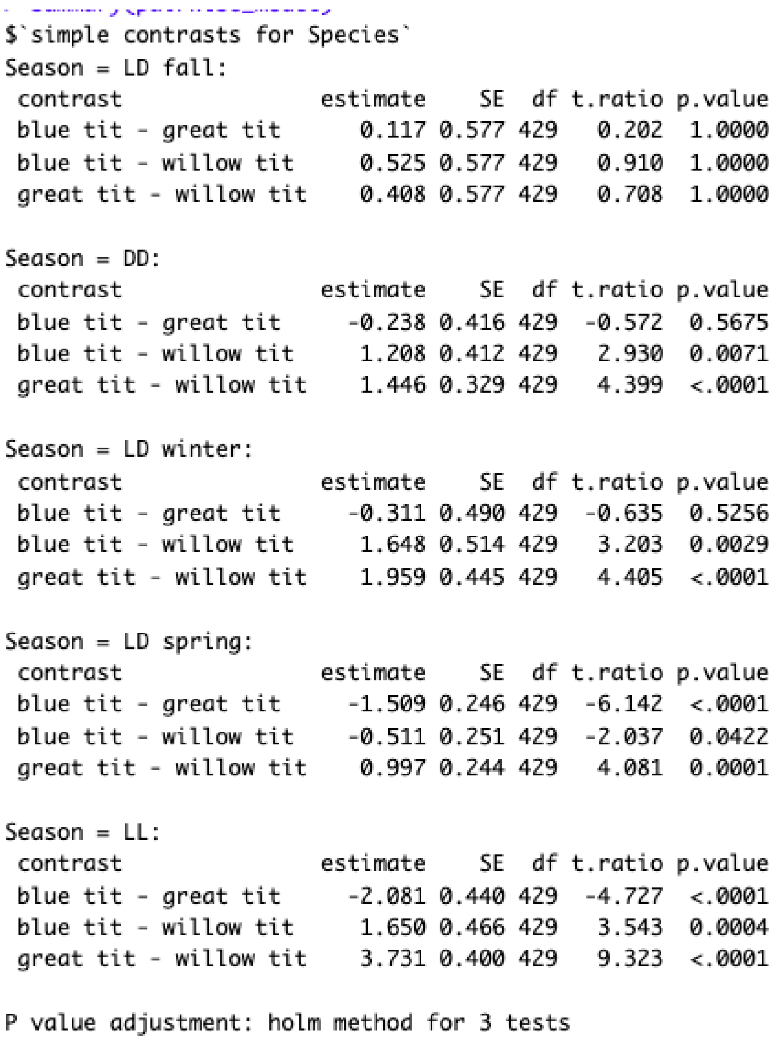


Figure C - 3: The seasonal change in onset of activity relative to nautical dawn (grey line) in hours. Species are represented with colour: blue tit (blue), great tit (red), and willow tit (green).

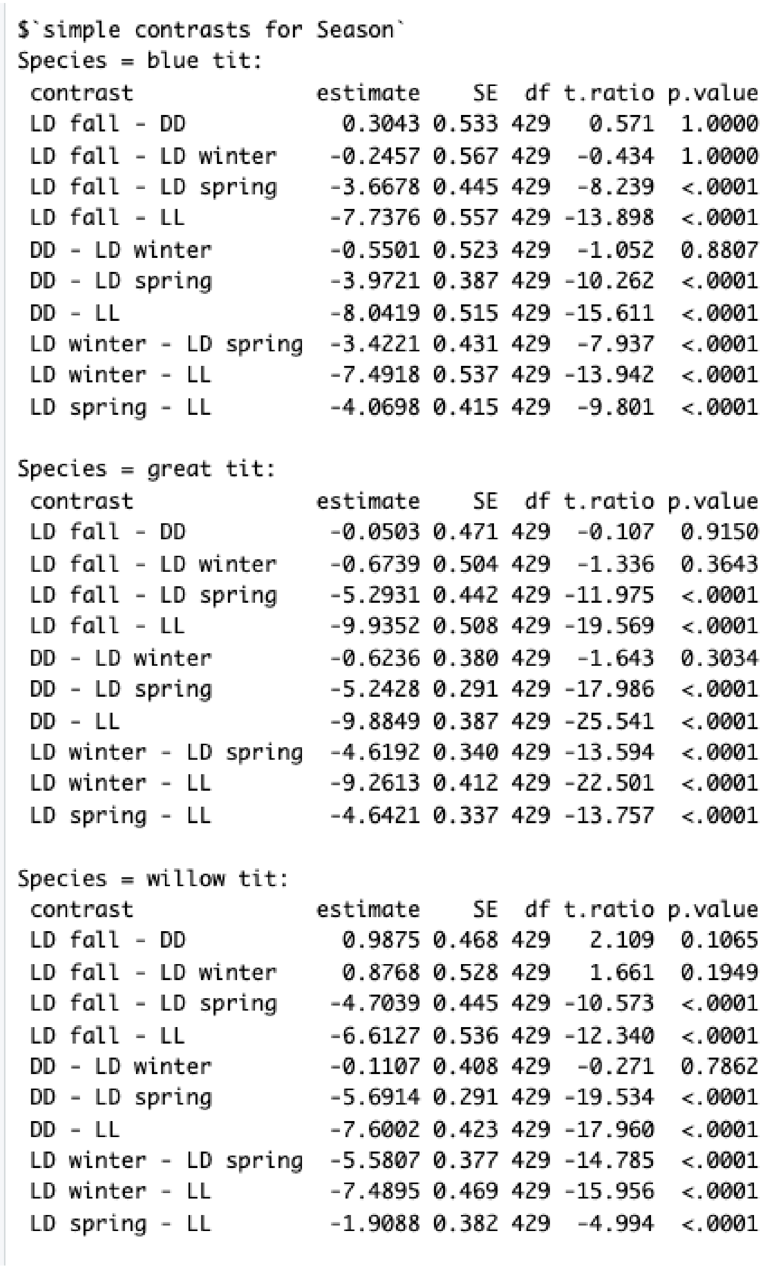
Appendix D: Supplementary Tables

Tukey post hoc test - Multiple pairwise comparisons



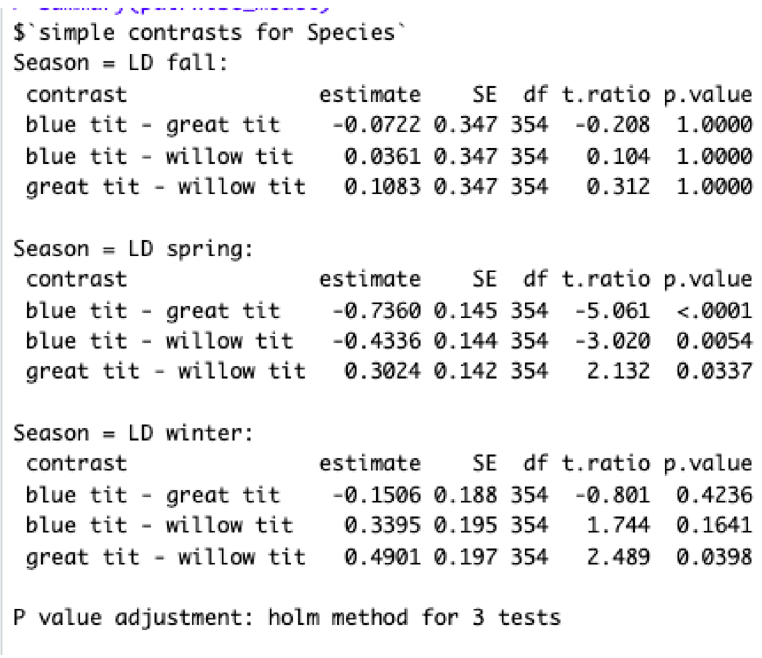
```
$`simple contrasts for Species`
Season = LD fall:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit       0.117 0.577 429   0.202  1.0000
 blue tit - willow tit      0.525 0.577 429   0.910  1.0000
 great tit - willow tit     0.408 0.577 429   0.708  1.0000

Season = DD:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      -0.238 0.416 429  -0.572  0.5675
 blue tit - willow tit      1.208 0.412 429   2.930  0.0071
 great tit - willow tit     1.446 0.329 429   4.399  <.0001

Season = LD winter:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      -0.311 0.490 429  -0.635  0.5256
 blue tit - willow tit      1.648 0.514 429   3.203  0.0029
 great tit - willow tit     1.959 0.445 429   4.405  <.0001

Season = LD spring:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      -1.509 0.246 429  -6.142  <.0001
 blue tit - willow tit     -0.511 0.251 429  -2.037  0.0422
 great tit - willow tit     0.997 0.244 429   4.081  0.0001

Season = LL:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      -2.081 0.440 429  -4.727  <.0001
 blue tit - willow tit      1.650 0.466 429   3.543  0.0004
 great tit - willow tit     3.731 0.400 429   9.323  <.0001

P value adjustment: holm method for 3 tests
```

Table D-1: Tukey Post Hoc – Mean differences in activity time and standard error in hours between species at the same season.



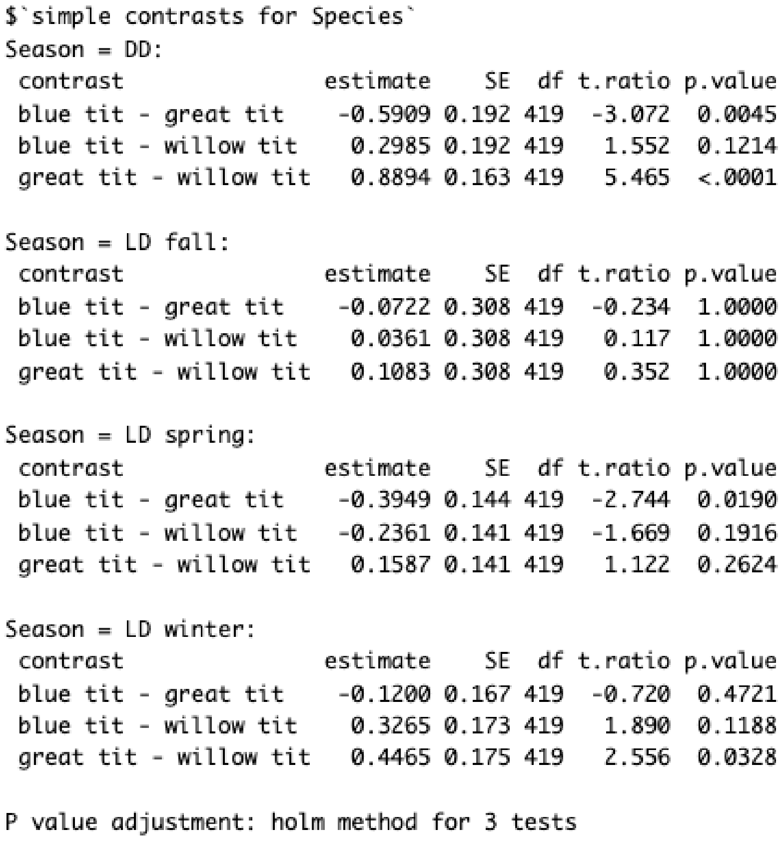
```
$`simple contrasts for Season`
Species = blue tit:
 contrast              estimate    SE  df t.ratio p.value
 LD fall - DD            0.3043 0.533 429   0.571  1.0000
 LD fall - LD winter    -0.2457 0.567 429  -0.434  1.0000
 LD fall - LD spring    -3.6678 0.445 429  -8.239  <.0001
 LD fall - LL           -7.7376 0.557 429 -13.898  <.0001
 DD - LD winter         -0.5501 0.523 429  -1.052  0.8807
 DD - LD spring         -3.9721 0.387 429 -10.262  <.0001
 DD - LL                -8.0419 0.515 429 -15.611  <.0001
 LD winter - LD spring  -3.4221 0.431 429  -7.937  <.0001
 LD winter - LL         -7.4918 0.537 429 -13.942  <.0001
 LD spring - LL         -4.0698 0.415 429  -9.801  <.0001

Species = great tit:
 contrast              estimate    SE  df t.ratio p.value
 LD fall - DD           -0.0503 0.471 429  -0.107  0.9150
 LD fall - LD winter    -0.6739 0.504 429  -1.336  0.3643
 LD fall - LD spring    -5.2931 0.442 429 -11.975  <.0001
 LD fall - LL           -9.9352 0.508 429 -19.569  <.0001
 DD - LD winter         -0.6236 0.380 429  -1.643  0.3034
 DD - LD spring         -5.2428 0.291 429 -17.986  <.0001
 DD - LL                -9.8849 0.387 429 -25.541  <.0001
 LD winter - LD spring  -4.6192 0.340 429 -13.594  <.0001
 LD winter - LL         -9.2613 0.412 429 -22.501  <.0001
 LD spring - LL         -4.6421 0.337 429 -13.757  <.0001

Species = willow tit:
 contrast              estimate    SE  df t.ratio p.value
 LD fall - DD            0.9875 0.468 429   2.109  0.1065
 LD fall - LD winter     0.8768 0.528 429   1.661  0.1949
 LD fall - LD spring    -4.7039 0.445 429 -10.573  <.0001
 LD fall - LL           -6.6127 0.536 429 -12.340  <.0001
 DD - LD winter         -0.1107 0.408 429  -0.271  0.7862
 DD - LD spring         -5.6914 0.291 429 -19.534  <.0001
 DD - LL                -7.6002 0.423 429 -17.960  <.0001
 LD winter - LD spring  -5.5807 0.377 429 -14.785  <.0001
 LD winter - LL         -7.4895 0.469 429 -15.956  <.0001
 LD spring - LL         -1.9088 0.382 429  -4.994  <.0001
```

Table D-2: Tukey Post Hoc – Mean differences in activity time and standard error in hours between season for each species.



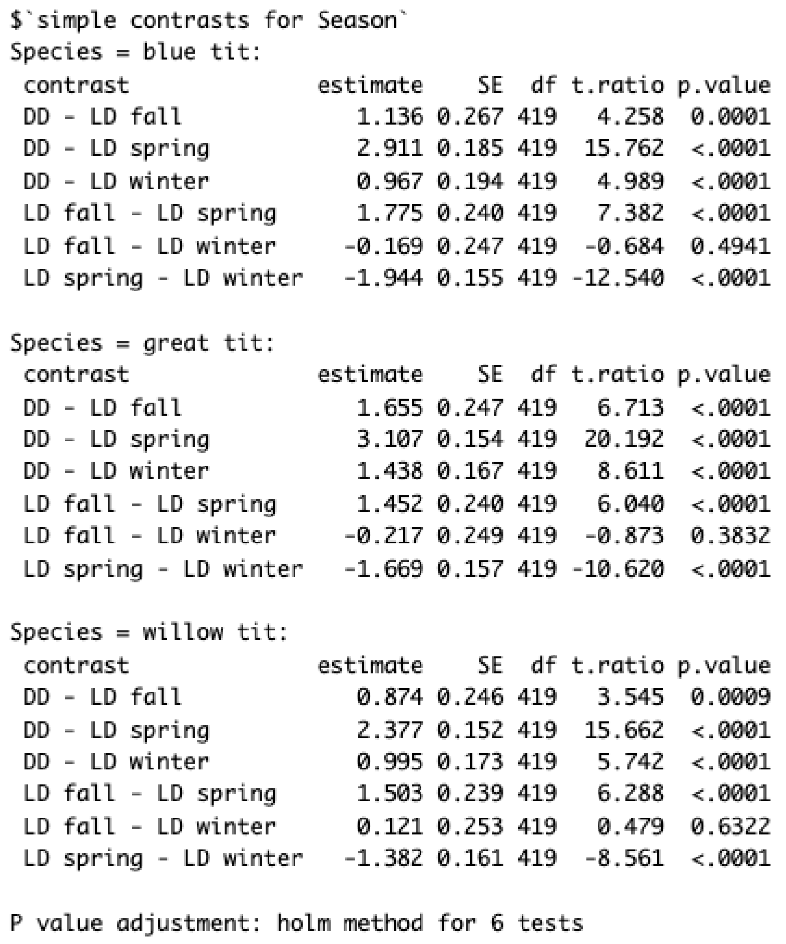
```
$`simple contrasts for Species`
Season = LD fall:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.0722 0.347 354  -0.208  1.0000
 blue tit - willow tit    0.0361 0.347 354   0.104  1.0000
 great tit - willow tit   0.1083 0.347 354   0.312  1.0000

Season = LD spring:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.7360 0.145 354  -5.061  <.0001
 blue tit - willow tit   -0.4336 0.144 354  -3.020  0.0054
 great tit - willow tit   0.3024 0.142 354   2.132  0.0337

Season = LD winter:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.1506 0.188 354  -0.801  0.4236
 blue tit - willow tit    0.3395 0.195 354   1.744  0.1641
 great tit - willow tit   0.4901 0.197 354   2.489  0.0398

P value adjustment: holm method for 3 tests
```

Table D-3: Tukey Post Hoc – Mean differences in Ψ-onset using sunrise as phase mark and standard error in hours between species for each season.



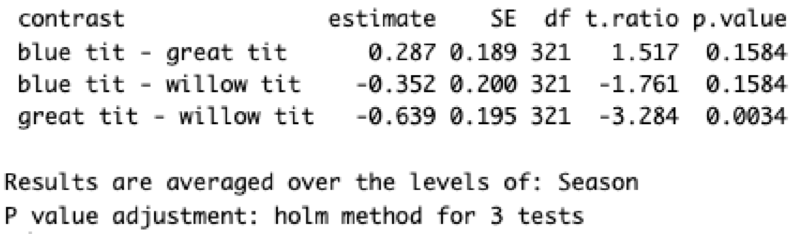
```
$`simple contrasts for Species`
Season = DD:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.5909 0.192 419  -3.072  0.0045
 blue tit - willow tit    0.2985 0.192 419   1.552  0.1214
 great tit - willow tit   0.8894 0.163 419   5.465  <.0001

Season = LD fall:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.0722 0.308 419  -0.234  1.0000
 blue tit - willow tit    0.0361 0.308 419   0.117  1.0000
 great tit - willow tit   0.1083 0.308 419   0.352  1.0000

Season = LD spring:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.3949 0.144 419  -2.744  0.0190
 blue tit - willow tit   -0.2361 0.141 419  -1.669  0.1916
 great tit - willow tit   0.1587 0.141 419   1.122  0.2624

Season = LD winter:
 contrast               estimate    SE  df t.ratio p.value
 blue tit - great tit    -0.1200 0.167 419  -0.720  0.4721
 blue tit - willow tit    0.3265 0.173 419   1.890  0.1188
 great tit - willow tit   0.4465 0.175 419   2.556  0.0328

P value adjustment: holm method for 3 tests
```

Table D-4: Tukey Post Hoc – Mean differences in Ψ-onset using civil dawn as phase mark and standard error in hours between species for each season.



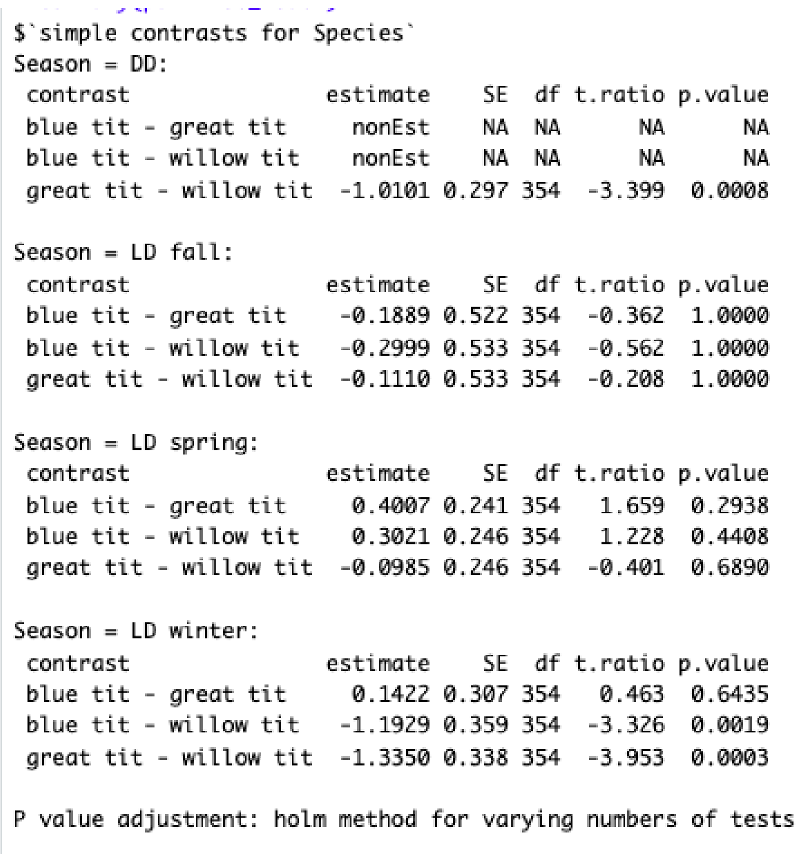
```
$`simple contrasts for Season`
Species = blue tit:
 contrast              estimate    SE  df t.ratio p.value
 DD - LD fall             1.136 0.267 419   4.258  0.0001
 DD - LD spring           2.911 0.185 419  15.762  <.0001
 DD - LD winter           0.967 0.194 419   4.989  <.0001
 LD fall - LD spring      1.775 0.240 419   7.382  <.0001
 LD fall - LD winter     -0.169 0.247 419  -0.684  0.4941
 LD spring - LD winter   -1.944 0.155 419 -12.540  <.0001

Species = great tit:
 contrast              estimate    SE  df t.ratio p.value
 DD - LD fall             1.655 0.247 419   6.713  <.0001
 DD - LD spring           3.107 0.154 419  20.192  <.0001
 DD - LD winter           1.438 0.167 419   8.611  <.0001
 LD fall - LD spring      1.452 0.240 419   6.040  <.0001
 LD fall - LD winter     -0.217 0.249 419  -0.873  0.3832
 LD spring - LD winter   -1.669 0.157 419 -10.620  <.0001

Species = willow tit:
 contrast              estimate    SE  df t.ratio p.value
 DD - LD fall             0.874 0.246 419   3.545  0.0009
 DD - LD spring           2.377 0.152 419  15.662  <.0001
 DD - LD winter           0.995 0.173 419   5.742  <.0001
 LD fall - LD spring      1.503 0.239 419   6.288  <.0001
 LD fall - LD winter      0.121 0.253 419   0.479  0.6322
 LD spring - LD winter   -1.382 0.161 419  -8.561  <.0001

P value adjustment: holm method for 6 tests
```

Table D-5: Tukey Post Hoc – Mean differences in Ψ-onset using civil dawn and standard error in hours between season for each species.



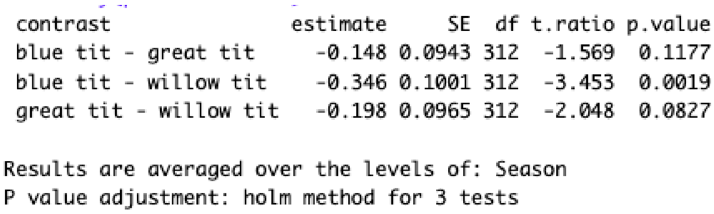
```
 contrast                 estimate    SE  df t.ratio p.value
 blue tit - great tit        0.287 0.189 321   1.517  0.1584
 blue tit - willow tit      -0.352 0.200 321  -1.761  0.1584
 great tit - willow tit     -0.639 0.195 321  -3.284  0.0034

Results are averaged over the levels of: Season
P value adjustment: holm method for 3 tests
```

Table D-6: Tukey Post Hoc – Mean differences in Ψ-offset using sunset as phase mark and standard error in hours between species.



```
$`simple contrasts for Species`
Season = DD:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      nonEst    NA  NA      NA      NA
 blue tit - willow tit     nonEst    NA  NA      NA      NA
 great tit - willow tit   -1.0101 0.297 354  -3.399  0.0008

Season = LD fall:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit     -0.1889 0.522 354  -0.362  1.0000
 blue tit - willow tit    -0.2999 0.533 354  -0.562  1.0000
 great tit - willow tit   -0.1110 0.533 354  -0.208  1.0000

Season = LD spring:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      0.4007 0.241 354   1.659  0.2938
 blue tit - willow tit     0.3021 0.246 354   1.228  0.4408
 great tit - willow tit   -0.0985 0.246 354  -0.401  0.6890

Season = LD winter:
 contrast                estimate    SE  df t.ratio p.value
 blue tit - great tit      0.1422 0.307 354   0.463  0.6435
 blue tit - willow tit    -1.1929 0.359 354  -3.326  0.0019
 great tit - willow tit   -1.3350 0.338 354  -3.953  0.0003

P value adjustment: holm method for varying numbers of tests
```

Table D-7: Tukey Post Hoc – Mean differences in Ψ-offset using civil dusk as phase mark and standard error in hours between species for each season.



```
 contrast               estimate     SE  df t.ratio p.value
 blue tit - great tit     -0.148 0.0943 312  -1.569  0.1177
 blue tit - willow tit    -0.346 0.1001 312  -3.453  0.0019
 great tit - willow tit   -0.198 0.0965 312  -2.048  0.0827

Results are averaged over the levels of: Season
P value adjustment: holm method for 3 tests
```

Table D-8: Tukey Post Hoc – Mean differences in Ψ-midpoint using civil dusk as phase mark and standard error in hours between species.

Appendix E: Challenges - feeder-camera set up

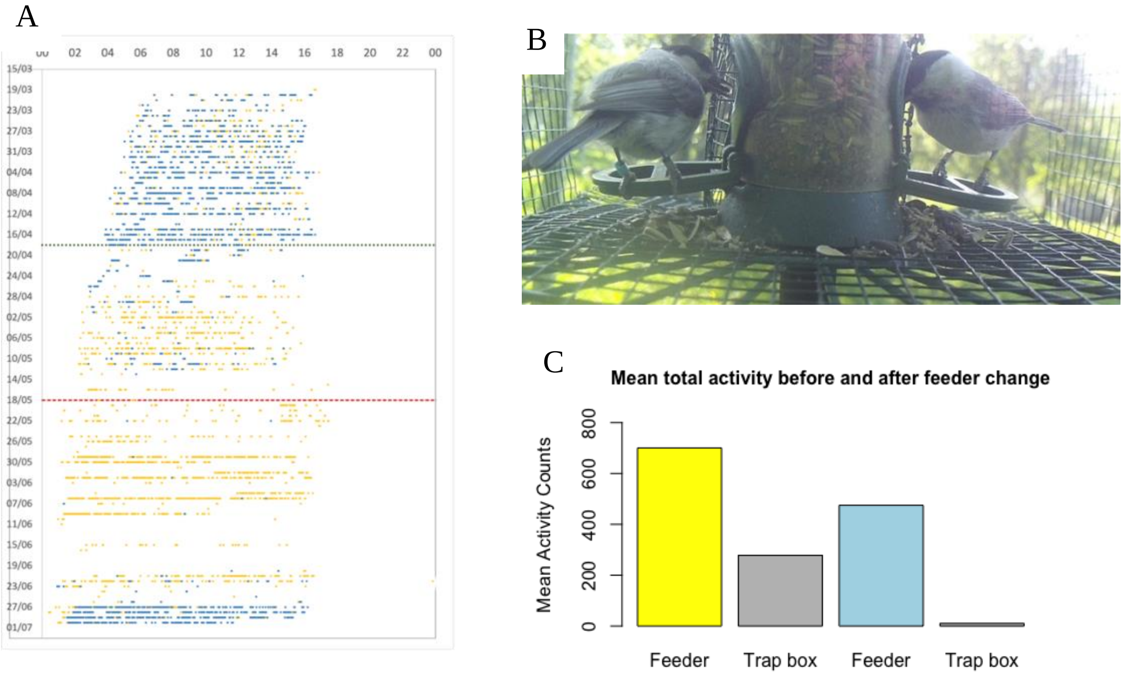


Figure E - 1. A. Single-plotted actogram of two different willow tits: WT_ye (yellow points) and WT_lb (blue points). Green line marks when we change to trap box and red line is when feeder 3 was removed. **B.** Picture of WT-ye and WT_lb in the trap box. **C.** Mean total activity before changing the feeder to trap box for WT_ye (yellow) and WT_lb (blue). Mean total activity after changing to a trap box (grey). The activity counts are only from feeder 2. The units are reported per day in 10-day intervals.

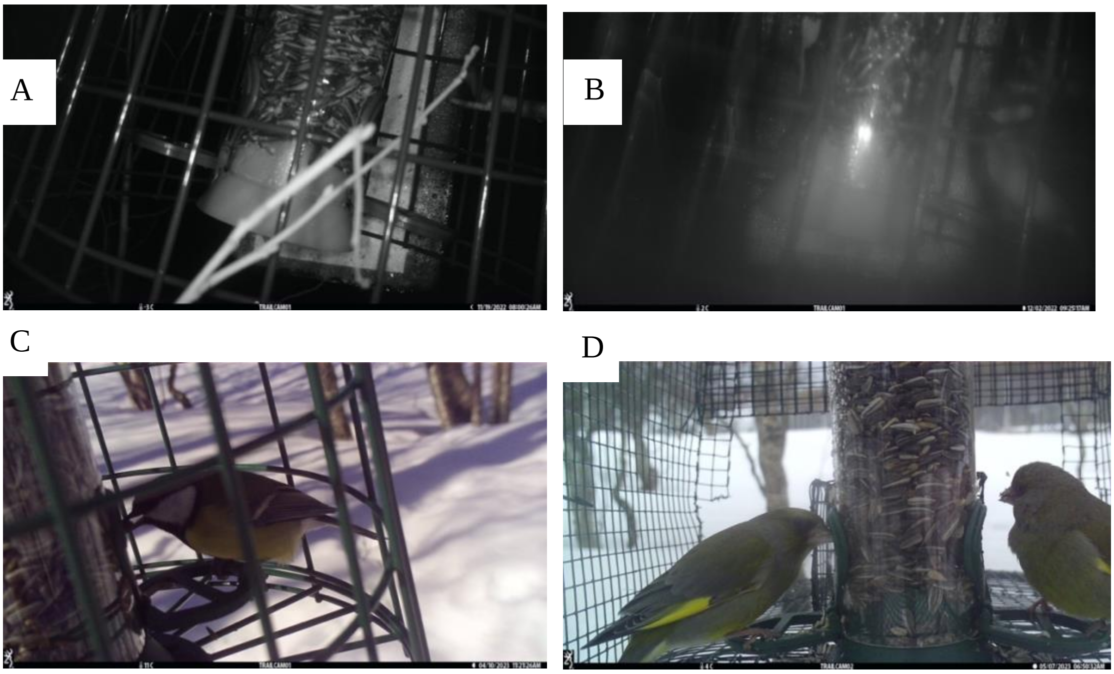


Figure E – 2: Examples of situations leading poor quality images and increased amounts of images. A. something in front of the lens triggering the camera. **B.** lens condensation due to high air humidity, difficult to distinguish the bird species. **C.** Feeder tilted due to snow melt during spring, only one chamber is visible. **D.** Other bird species than the tits dominating the feeders such as the green finches.

Appendix F: Other behavioural activities

F – 1: Method

In addition to the foraging behaviour, the primary focus of this study, I also collected data during the breeding season of nesting and egg laying behaviour and behaviour at the nest box during incubation and chick rearing. I placed 85 nest boxes throughout the study period with approximately 100 meters distance from each other and approximately 2 meter above the ground (appendix map..). 35 nest boxes were from Biltema and placed in November, 2022. Later, 50 nest boxes were provided by Wildlab Projects in connection with the Citizen Science Project on how light pollution affect songbirds, which is a project that UiT and WildLab Projects are collaborating on. These nest boxes are more solid than the Biltema ones, and also, they are made of reused pallets, which contribute to environmental sustainability by minimizing waste. The study area for the breeding season 2023 was complete May 3rd.

We started to monitor the nest boxes from late April, in anticipation of the breeding season onset, to the end of July. Each nest box underwent checks at least every 10 days. As the breeding season progressed, detailed records were maintained, capturing nest stage, egg count (with temperature distinctions of cold or warm), hatching date, chick count and age, and the fledging date. This made it possible measure behaviour rhythms such as incubation and nest box activity, which was only measured for great tits due to the absence of successful blue tit nests.

Nest box activity

Nest box activity for great tits was assessed using a camera trap, using the same methodology as for monitoring feeder visits. The camera trap was placed at nearby tree or branch without the custom-made lenses the distance between the nest box and camera-trap was approximately 1 – 1.5 meter. A camera trap was placed at nest box 1, 5 and 37. It was not possible to distinguish female nest box activity from the male, therefore they are handled together. The SD cards were changed every second day.

Incubation activity

To measure incubation activity, we placed temperature loggers (ibuttons) within the nest cup. The ibuttons record temperature and tell us when the female is inside the nest incubating or not. The sample rate of the ibutton was set to every 180 seconds and temperature logging was set to 0.5 °C. Great tits remove ibuttons from nest boxes, and thus the devices need to be secured in place. We used a malleable piece of wire for that purpose similar to what is done in other locations (Tomotani et al, 2023). However, the composition of the nests of Tromsø birds made the placement harder (thick layer of hair and loose layer of moss). In order to avoid nest disruption and reduce the risk of egg abandonment by the female we decided to stop the placement of ibuttons. As a result, ibutton loggers were only placed in four nest boxes, numbered 1, 5, 7 and 33, occupied of great tits. The ibuttons were placed in the end of the egg-laying phase and right before the incubation phase. The ibutton loggers started to sample May 23rd and ended June 9th.

F - 2: Preliminary Data

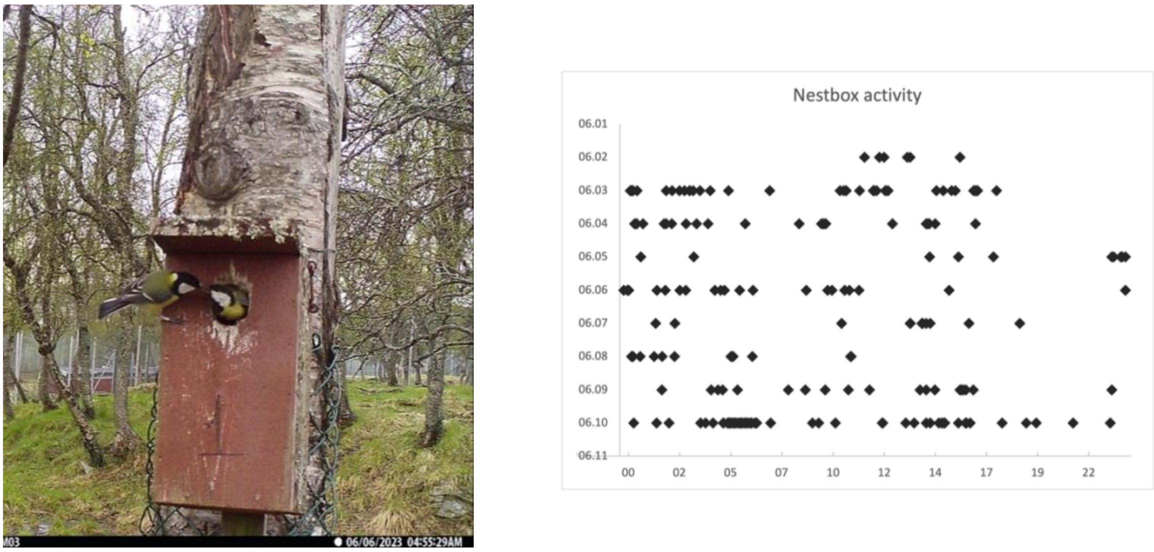


Figure F – 2.1: Activity at nest box 1 of A53 and A54. **A**. Male great tit (A53) feeding female great tit (A54) at their nest. **B.** Actogram illustrating nest activity by A53 and A54. Black dots illustrate when they have been observed at nest box.

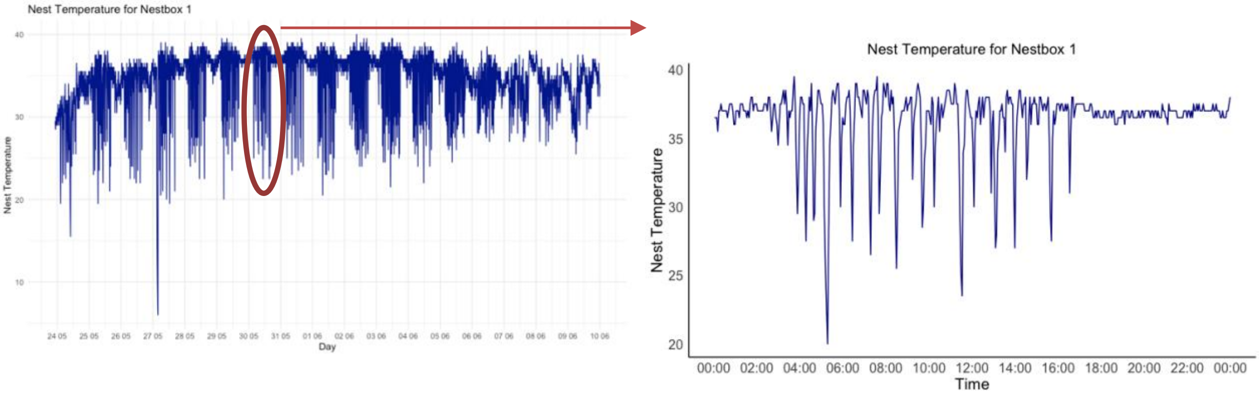


Figure F – 2.2: Incubation activity of A54 provided by ibutton loggers at nest box 1. Temperature on y-axis and time of day on x-axis.

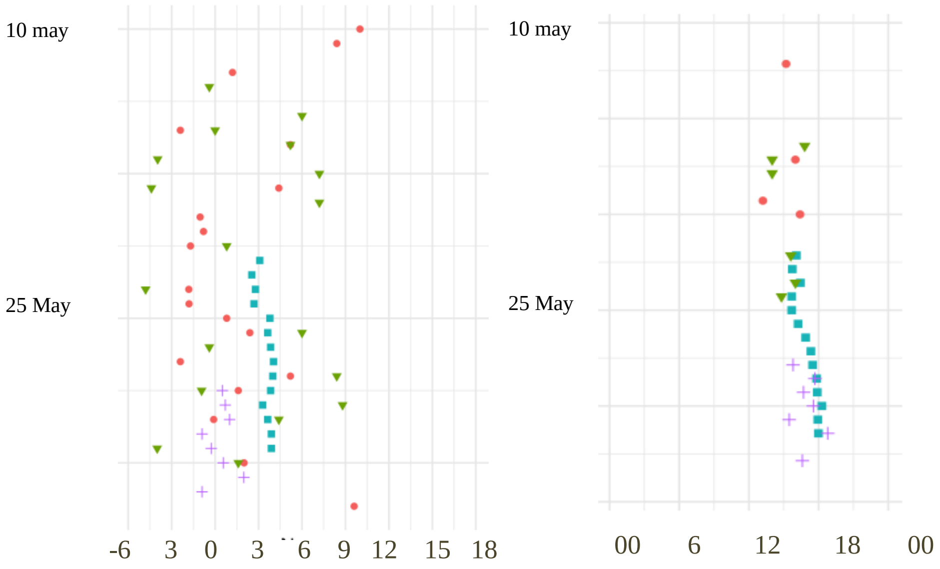


Figure F – 2.3: Onset and offset of activity of three different behaviours; visit at the feeders, nest box activity and incubation. Left: onset of activity of the different behaviours, visit at feeders for A53 (red points) and A54 (green triangles), nest box activity (purple cross) and incubation activity (blue square). X-axis represent time where 0 is the time of midnight, - is hours before midnight. **Right:** offset of activity, x- axis represent the time of day.

Appendix G: R-codes

Multiple linear regression model

```
model_lm_phase_sunrise <- lm(phase_angle_sunrise ~ Species:Season + Species + Season + average.temp,
             data = ActivityData_filtered)

# find p-values

drop1(model_lm_phase_sunrise, test="F")
```

Tukey post hoc test

```
# Calculate estimated marginal means

emm_onset_sunrise <- emmeans(model_lm_phase_sunrise, ~ Species:Season)

summary(emm_onset_sunrise)

# Conduct pairwise comparisons

pairwise_model <- pairs(emm_onset_sunrise, simple = “each”, adjust="holm")

summary(pairwise_model)
```

The same R codes was used for all the analysis, which of independent variables included in the model varied for between the analysis due to interaction was not always