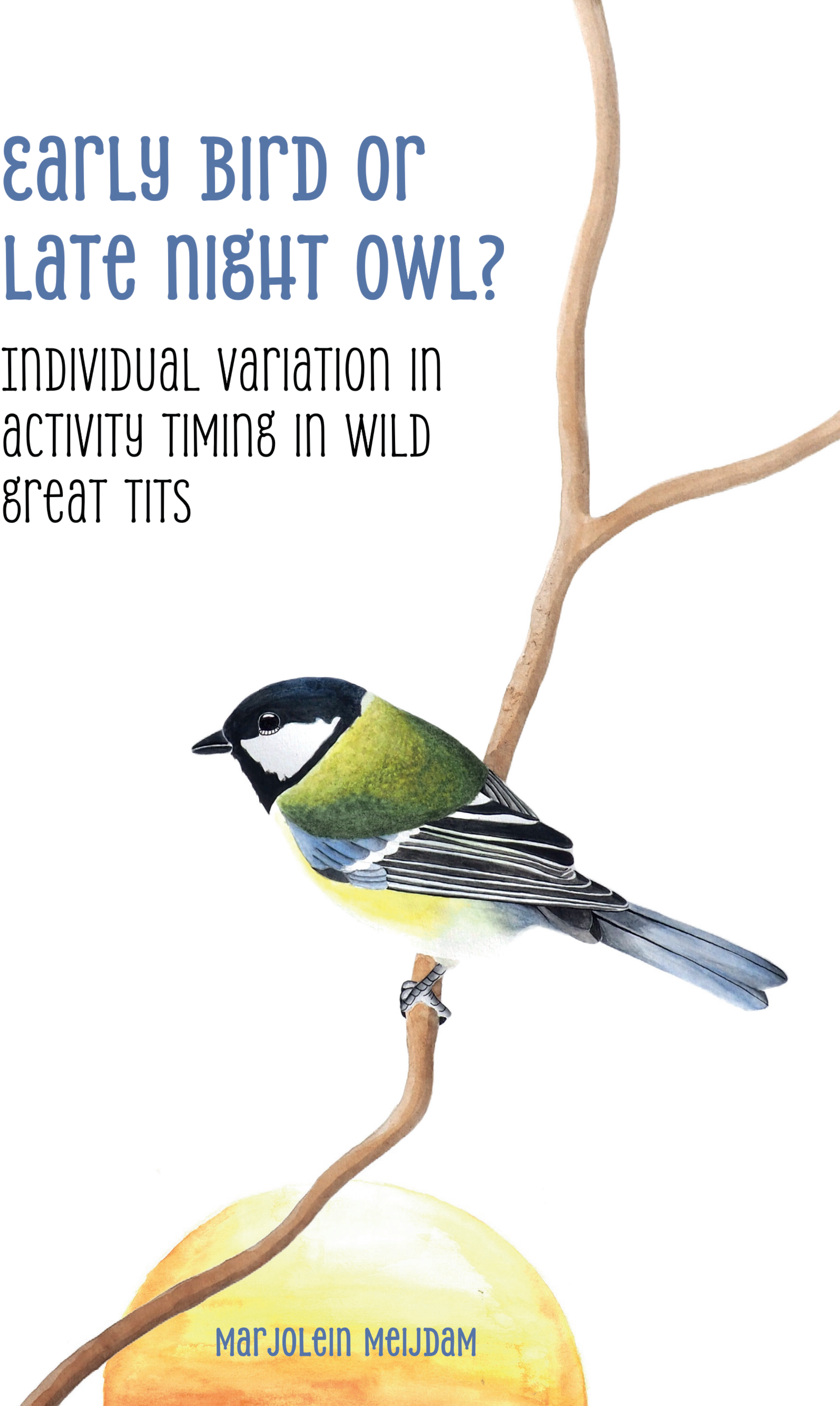
Early Bird or Late Night Owl?

Individual variation in activity timing in wild great tits



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PhD thesis submitted for the degree of Doctor in Science: Biology

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The research presented in this thesis was carried out at the Behavioural Ecology and Ecophysiology Research Group at the University of Antwerp

The preferred citation for this thesis is:

Meijdam, M. (2024) Early bird or late night owl? Individual variation in activity timing in wild great tits. PhD thesis, University of Antwerp, Antwerp, Belgium.

Cover illustration: Marjolein Meijdam

This research was funded by the University of Antwerp

Universiteit
Antwerpen

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Summary

Many behavioural and physiological processes fluctuate throughout the day. Such circadian rhythms are predominantly regulated internally by the molecular clock, a negative feedback loop that controls gene expression roughly in a 24 hour schedule. Circadian rhythms are in addition modulated by external factors such as light and temperature (i.e. Zeitgebers) and activity rhythms can be strongly affected by masking factors, which directly affect the timing of behaviour without interacting with the circadian clock. Differences between individuals in the precise duration of the feedback loop or differences in sensitivity to Zeitgebers and masking factors can cause individuals to exhibit differences in activity rhythms, with some individuals tending to be generally active earlier in the day than others. An individual's temporal phenotype is also referred to as its chronotype and individual differences in chronotype are increasingly being reported in free-living species in a variety of taxa. Although the evolutionary history of the development of these differences in chronotype occurred mainly in natural conditions, many habitats today are disturbed by anthropogenic stressors, such as light, noise and chemical pollution, that can affect the timing of activity. In order to understand the possible effects of such stressors on wildlife, it is therefore of utmost importance to first increase our understanding of the functional consequences of differences in chronotypes as well as how and why variation in this trait occurs, as this has remained a mystery to date. Next, it is necessary to investigate how individual activity rhythms are affected by anthropogenic disturbances and what the possible consequences of such disturbances might be. To this end, I studied individual activity rhythms of great tits (*Parus major*) in a suburban population, by determining their timing of activity onset in the morning and activity offset in the evening.

First, I investigated whether adult great tits show individual differences in the timing of activity onset and whether that is consistent even over longer periods (i.e. between years). Indeed both individual variation and long term consistency were present in my

study population, indicating the presence of chronotypes, which formed the basis of my subsequent studies. Still, individuals also appeared to show some plasticity in their timing, especially on temporal scales, while differences in environmental conditions between different spatial locations (e.g. light pollution or habitat quality) appeared to have no effect on timing.

Yet, how is population level variation in chronotypes maintained and what are the individual costs and benefits that determine the right time to be active? At dawn and dusk, for example, predation risk or the risk of hypothermia may be higher, while competition for food is likely to be lower. Differences between individuals in the timing of their behaviour can thus create differences in exposure to risks and opportunities, which can be important for fitness. Therefore, I examined for both females and males whether the timing of activity was related to reproductive success and survival. The timing of the start of activity had no influence in both sexes, but in males, later timing of activity offset was related to lower reproductive success and higher mortality. Even though the causality is unknown, this suggests that anthropogenic stressors, of which it is known that they can delay the timing of activity offset, could have negative fitness consequences for males.

However, selection for certain chronotypes does not only depend on direct effects of the timing of activity on fitness, but may also be affected by associations with other traits. For example, evidence for an association between the circadian clock and the annual timing of reproduction has been found. However, I found no correlation between daily timing of activity and timing of reproduction in this thesis, and constraints on the evolution of both traits through interdependence is thus unlikely. Thus while the timing of reproduction has greatly advanced in recent decades due to climate change, this did not necessarily impact on the daily timing of activity. However, I did find a link between chronotype and a personality trait: aggression. While I expected individuals with low aggression levels to have earlier chronotypes, enabling foraging early at low social densities to minimise competition, the relationship between

chronotype and aggression was actually the opposite. Females with early chronotypes were more aggressive, yet whether this is adaptive needs to be shown. Possibly, this association could reflect shared underlying mechanisms, such as genetic or hormonal pleiotropy, affecting both traits, which would hinder their independent evolution and could result in suboptimal behaviour.

Although no strong effects of anthropogenic disturbance on the timing of activity were found in this thesis, there are indications that this could be due to the shielding effect of nest boxes, and that at other times of the year, when great tits do not sleep in the nest box, these effects could be stronger. Such changes in activity patterns could also affect the amount of sleep an individual gets. Therefore, as a proof of principle, I investigated the effect of sleep deprivation on cognitive functions, as has been shown in humans (among others). After exposure to artificial light at night, great tits showed decreased impulse control, but their vigilance was not affected. Such deteriorated cognitive performance may have adverse fitness consequences, especially when exposure to sleep-disrupting stressors is prolonged. However, as mentioned above, this may only affect great tits when sleeping outside of their nest box, and it may be that individuals adapt to such disturbance over time.

Altogether, this thesis has provided data that are needed to understand how selection can act on the timing of activities, i.e. chronotypes. It not only highlights the functional consequences of differences between individuals in the timing of behaviour, but it also emphasizes how anthropogenic stressors could impinge on urban birds. This knowledge could be of great importance for a better understanding of the key processes of the impact of urbanization on wildlife and to develop sustainable urban planning frameworks.

Samenvatting

Veel gedrags- en fysiologische processen schommelen gedurende de dag. Zulke circadiane ritmes worden hoofdzakelijk intern gereguleerd door de moleculaire klok, een negatieve feedbackloop die ongeveer 24 uur duurt en genexpressie controleert. Daarnaast worden circadiane ritmes gemoduleerd door externe factoren zoals licht en temperatuur (i.e. Zeitgebers) en kunnen activiteit ritmes sterk beïnvloed worden door maskerende factoren die de timing van gedrag direct beïnvloeden zonder interactie met de circadiane klok. Verschillen tussen individuen in de precieze duur van de feedbackloop of verschillen in de gevoeligheid voor Zeitgebers en maskerende factoren kunnen ervoor zorgen dat individuen verschillen vertonen in activiteit ritmes, waarbij sommige individuen geneigd zijn over het algemeen vroeger op de dag actief te zijn dan anderen. Een individu zijn temporele fenotype wordt ook wel aangeduid met de term chronotype en individuele verschillen in chronotype worden steeds vaker gerapporteerd bij in het wild levende soorten. Hoewel de evolutionaire geschiedenis van de ontwikkeling van verschillen in chronotype zich voornamelijk heeft voltrokken in natuurlijke omstandigheden, zijn veel habitats tegenwoordig verstoord door antropogene stressoren die de timing van activiteit kunnen beïnvloeden, zoals licht-, geluid- en chemische vervuiling. Om te kunnen begrijpen welke mogelijke effecten dit heeft op wilde dieren is het daarom van uiterst belang om eerst meer inzicht te verwerven in de functionele consequenties van verschillen in chronotypes en hoe en waarom de variatie in dit kenmerk in stand wordt gehouden, aangezien dit tot op heden een mysterie is gebleven. Vervolgens is het noodzakelijk te onderzoeken hoe de individuele activiteit ritmes beïnvloed worden door antropogene verstoring en wat daarvan de mogelijke consequenties zijn. Hiertoe heb ik de individuele activiteit ritmes onderzocht van koolmezen in een voorstedelijke populatie.

Eerst heb ik onderzocht of individuen in mijn populatie verschillen vertoonden in de timing van activiteit en of die ook over langere periodes (i.e. tussen jaren) consistent

bleven, waarbij ik mij focuste op de timing van de start van activiteit in de ochtend. Dit blek inderdaad het geval, wat duidt op de aanwezigheid van verschillen in chronotype en als basis dient voor mijn vervolgstudies. Toch bleken individuen ook plasticiteit te vertonen in hun timing, waarbij vooral veranderingen op temporele schaal een rol speelden, terwijl verschillen in omgevingsomstandigheden tussen verschillende spatiele locaties (e.g. lichtvervuiling of habitatkwaliteit) geen effect op de timing had.

Maar hoe wordt variatie in chronotypes op populatieniveau in stand gehouden en wat zijn de individuele kosten en baten die het juiste moment om actief te zijn bepalen? In de schemering kunnen predatierisico en het risico op onderkoeling bijvoorbeeld groter zijn, terwijl competitie voor voedsel waarschijnlijk juist lager is. Verschillen tussen individuen in de timing van hun gedrag kunnen dus zorgen voor verschillen in blootstelling aan risico's en opportuniteiten, die belangrijk kunnen zijn voor fitness. Daarom heb ik voor zowel vrouwtjes als mannetjes onderzocht hoe de timing van activiteit samenhangt met reproductief succes en overleving. De timing van de start van activiteit bleek hier in beide geslachten geen invloed op te hebben, maar voor mannetjes was een latere timing van het beëindigen van activiteit gerelateerd aan lager reproductief succes en hogere mortaliteit. Hoewel de causaliteit van dit verband nog onbekend is, lijkt het erop te wijzen dat antropogene stressoren, waarvan bekend is dat ze het tijdstip van de beëindiging van activiteit kunnen uitstellen, negatieve effecten op de fitness van mannetjes kunnen hebben.

Selectie voor bepaalde chronotypes hangt echter niet enkel af van directe gevolgen van de timing van activiteit voor fitness, maar kan ook worden beïnvloed door associaties met andere kenmerken. Zo bestaat er een samenhang tussen de circadiane klok en de jaarlijkse timing van reproductie. Ik vond in deze thesis echter geen verband tussen de dagelijkse timing van activiteit en de timing van reproductie, en beperkingen voor de evolutie van beide kenmerken door onderlinge afhankelijkheid is dus onwaarschijnlijk. Hoewel de jaarlijkse timing van reproductie door klimaatverandering sterk vervroegd is in de laatste decennia, heeft dit dus waarschijnlijk geen impact gehad op de dagelijkse timing van activiteit. Ik vond echter wel een verband tussen chronotype en een persoonlijkheidskenmerk: agressie. Hoewel ik verwacht had dat individuen die weinig agressief zijn een vroeger chronotype zouden hebben om vroeg te kunnen foerageren bij lage sociale densiteit en zo competitie te minimaliseren, bleek het verband tussen chronotype en agressie juist omgekeerd. Vrouwtjes met vroege chronotypes waren agressiever, maar of dit adaptief is moet nog blijken. Deze associatie zou mogelijks kunnen worden veroorzaakt door gedeelde onderliggende mechanismen die beide kenmerken beïnvloeden, zoals genetische of hormonale pleiotropie, wat de onafhankelijke evolutie van beide kenmerken kan verhinderen en kan resulteren in suboptimaal gedrag.

Hoewel in deze thesis geen sterke effecten van antropogene verstoring op de timing van activiteit werden gevonden, zijn er aanwijzingen dat dit komt door het afschermende effect van nestkasten, en dat in andere periodes van het jaar, wanneer koolmezen niet in de nestkast slapen, deze effecten sterker zouden kunnen zijn. Zulke veranderingen in activiteitspatronen kunnen ook van invloed zijn op de hoeveelheid slaap die een individu krijgt. Daarom heb ik onderzocht wat het effect is van slaapdeprivatie op cognitieve vaardigheden, in navolging van resultaten bij (onder andere) mensen. Na een nacht blootstelling aan licht waren koolmezen inderdaad slechter in het controleren van hun impulsen, maar hun waakzaamheid werd niet beïnvloed. Zulke verslechterde cognitieve prestaties hebben mogelijk nadelige gevolgen voor fitness, zeker bij langdurige blootstelling aan slaap verstorende stressoren. Echter, zoals hierboven genoemd zou dit enkel van belang kunnen zijn voor koolmezen wanneer zij niet in de nestkast slapen. Bovendien kunnen individuen zich op termijn misschien aanpassen aan zulke verstoring.

Al met al heeft dit proefschrift data opgeleverd die nodig zijn om beter te kunnen begrijpen hoe selectie inwerkt op de timing van activiteit, i.e. chronotypes. Het belicht niet alleen de functionele consequenties van verschillen tussen individuen in de timing van gedrag, maar het benadrukt ook hoe antropogene stressoren nadelige effecten op stedelijke vogels kan hebben. Deze kennis kan essentieel zijn voor een beter begrip van de sleutelprocessen van de impact van urbanisatie op wilde dieren en voor de ontwikkeling van duurzame stedelijke planningskaders.

Chapter 1: General introduction

Our lives are a sequence of periods of activity and sleep, which alternate and follow cycles of about 24 hours, typically called circadian rhythms (in Latin, *circa* means ‘around’ and *dies* means ‘day’). Circadian rhythms are not unique to humans, as already in 1729 rhythmic daily leaf movements were reported in heliotropic plants (De Mairan, 1729). De Mairan noticed that in the morning the leaves moved up, while in the evening they drooped. These leaf movements persisted even when the plants were kept in a continuously dark environment, indicating that the observed daily rhythms were internally regulated. To test to what degree human circadian rhythms were internally regulated, a rather extreme experiment was conducted in 1938, when two researchers, Nathaniel Kleitman and Bruce Richardson, locked themselves for 32 days in a cave, isolated from any fluctuating environmental factors, such as light or temperature (Walker, 2017; Wolf-Meyer, 2013). They tried to test whether they could entrain their bodies to another rhythm than the 24 hour sleep/wake cycle. In order to do so, they used an alarm to set the rhythm at 28 hours, followed at strict sleeping schedule and to prevent rats and other cave dwelling creatures from waking them up, they placed large buckets around the bed legs. During the experiment they monitored their body temperatures and noted down how alert they felt. After 32 days Richardson had adjusted to the longer cycles, showing body temperature cycles of 28 hours and feeling alert during the active hours. However, Kleitman could not acclimate. His temperature cycles remained shorter than 28 hours and during the active hours he felt drowsy, while not being able to sleep during rest hours. Although their experiment included only two persons and the results are difficult to generalise, their results point to both the existence of circadian rhythms and the presence of individual differences therein, which are the central themes of my thesis.

Since the experiment by Kleitman and Richardson, chronobiologists have gained extensive insights in the internal mechanisms that regulate the circadian rhythm, called the circadian clock or biological clock. By internally keeping track of time and adjusting their behavioural and physiological rhythms accordingly, organisms may be able to anticipate predictable fluctuations in environmental variables, both biotic (e.g. food availability) and abiotic (e.g. light-dark cycles) (Deans, 2021). However, to predict such fluctuations correctly, it is essential that the circadian clock is sensitive to environmental variables, as the fluctuations may not be stable throughout the year. For example, light conditions differ between winter and summer. Indeed, many environmental variables, especially light and temperature, can entrain the circadian clock (Schwartz et al., 2017) and together they can affect the timing of activity. Interestingly, the timing of activity differs among species (e.g. Biggins et al., 2011; Bilu & Kronfeld-Schor, 2013; Stone et al., 1997), and as one might have suspected, it recently came to light that also within animal species individuals differ in the timing of activity (Alós et al., 2017; Chmura et al., 2020; Dominoni et al., 2013; Graham et al., 2017; M. L. Grunst et al., 2021; Maury et al., 2020; Roenneberg et al., 2007; Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015).

Sources of variation from a proximate perspective

The circadian clock

Individual variation in the timing of activity may relate to the proximate mechanisms that determine the timing of activity, and in the following section I will detail the general mechanisms underlying circadian rhythms. As the molecular and genetic tools available for the study of chronobiology in avian species were long inferior to those used for mammals and fruit flies, knowledge on the circadian clocks in avian species, to which my model species the great tit (*Parus major*) belongs, has long been lagged behind (Helm, 2020). Circadian clocks in birds and mammals show many similarities but the main brain areas involved differ. The avian circadian clock is a complex machinery that is orchestrated by several pacemakers throughout the body (Cassone et al., 2017). Pacemakers set the timing for physiological and behavioural processes, and they regulate rhythmicity of peripheral oscillators in tissues downstream. The main pacemakers in birds are the pineal gland, the retinae and the suprachiasmatic nuclei (SCN), but possibly additional unidentified pacemakers exist. The pacemakers can interact with each other, likely through the rhythmic secretion of the hormone melatonin, which has many binding sites throughout the avian brain (Fusani & Gahr, 2015; ViviD & Bentley, 2018). Potentially, other hormones, such as corticosterone, and neuromodulators like dopamine and norepinephrine are involved as well (Helm, 2020). The interaction between the different pacemakers amplifies and stabilises the internal rhythm, making it highly self-sustained (Gwinner & Brandstätter, 2001).

Within the pacemaker tissues, clock genes are rhythmically expressed. When the genes *Clock* and *bmal1* are transcribed, they can dimerize and stimulate the transcription of *period 2*, *period 3*, *cryptochrome 1* and *cryptochrome 2* by binding to E-box regulatory sequences in the promotor region of their DNA (Cassone et al., 2017). PER and CRY proteins form oligomers that inhibit the transcription of *Clock* and *bmal1*, thus forming a negative feedback loop. After a while, PER and CRY are degraded, the inhibition on *Clock* and *bmal1* is released and they will be transcribed once again. This cycle spans approximately 24 hours. In mammals, evidence is accumulating that many peripheral organs show such rhythmicity, even when the main pacemaker in mammals, the SCN, is not intact (van der Veen et al., 2017). In fact, molecular oscillators can be found in each nucleated cell throughout the body, and these molecular clocks potentially drive the dial rhythmic expression of thousands of different proteins, which can affect not only the timing of behaviours, such as wake-up time and sleep onset, but also physiological processes like hormone secretion, body temperature and immune response (Cassone, 2014; Markowska et al., 2017; Strauß et al., 2022). So, differences among individuals in the circadian rhythm may be determined by the individual's circadian clock properties.

One aspect of the circadian clock's properties that differs among individuals and that can explain differences in the timing of activity is the endogenous circadian period (τ), which is the duration of one full cycle of the circadian rhythm in the absence of any time giving cues. τ often slightly deviates from 24 hours and in mammals such individual differences in τ translate into differences in the phasing of activity among individuals, which is the timing of activity relative to an external temporal reference, for example light, under laboratory conditions, also called the chronotype (Brown et al., 2008; Pfeffer et al., 2015; Ralph & Menaker, 1988; Wicht et al., 2014). Individuals with early chronotypes tend to time their activity earlier during the day than individuals with late chronotypes. Although the chronotype can strictly only be measured under laboratory conditions, recently behavioural ecologists have started measuring the timing of activity in the wild and use the term to describe consistent variation among individuals in this trait (e.g. Alós et al., 2017; Dominoni et al., 2013; Maury et al., 2020; Womack et al., 2023a). This shift from the lab to natural conditions allows to study whether selection acts on the timing of activity. In this thesis I refer to this broader definition, i.e. the temporal phenotype, when studying chronotypes.

Zeitgebers, masking factors and human interference

Although the circadian clock maintains the circadian rhythm even in the absence of any cues, it can be attuned to the environment in natural conditions. Environmental factors that can entrain the circadian clock are called Zeitgebers. The main Zeitgeber in many species is light, but the input pathways and the relative importance of light sensitive tissues can differ among species (Helm, 2020). In birds, two pacemakers, the retina and the pineal gland (see above), are sensitive to light (Cassone et al., 2017; Menaker & Underwood, 1976; Underwood et al., 2001). Many other stimuli, both abiotic and biotic, can function as Zeitgeber and which Zeitgeber entrains the molecular clocks may be tissue specific. For example, although light is the main Zeitgeber for the pacemakers, in mammals the liver is entrained by food intake (van der Veen et al., 2017). Other potential Zeitgebers are temperature (Rensing & Ruoff, 2002; Sharma & Chandrashekaran, 2005), food availability (Hau & Gwinner, 1992; Stephan et al., 1979; van der Veen et al., 2011), social interactions (Castillo-Ruiz et al., 2012; Mistlberger &

Skene, 2004), predation risk (Bakker et al., 2005; Fenn & Macdonald, 1995) and competition (Sharma & Chandrashekaran, 2005).

Furthermore, in the wild many environmental factors directly affect the timing of behaviour without interacting with the circadian clock. For example, exposure to artificial light at night advances activity onset, but has no effect on the internal clock (Spoelstra et al., 2018). Removal of such environmental cues immediately restores the initial behavioural timing pattern (Rietveld et al., 1993; Schwartz et al., 2017). Such environmental factors are described as masking factors. Thus variation among individuals in the timing of activity may not only stem from differences in the circadian clock properties, but also from differences in sensitivity to Zeitgebers and masking factors (Helm et al., 2017; Jones et al., 2019; Womack et al., 2023a).

Intrinsic versus extrinsic factors

Together, the individual properties of the avian circadian clock and differences in sensitivity to Zeitgebers and masking factors determine the timing of activity of an individual (Figure 1). However, in the wild different individuals may be exposed to different abiotic and biotic environmental variables, as environmental variables may vary on spatial and temporal scales. Such variation in environmental variables to which individuals are exposed also causes variation among individuals in the timing of activity. For example, if the timing of activity onset in one individual is measured when a predator is present, while another individual is measured in the absence of predation risk, they are likely to show differences in behaviour that are extrinsically determined. Despite increasing evidence of inter-individual variation in the timing of activity in wild animals (Alós et al., 2017; Chmura et al., 2020; Dominoni et al., 2013; Graham et al., 2017; M. L. Grunst et al., 2021; Maury et al., 2020; Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015) it is unclear to what extent intrinsic factors, like the circadian clock and sensitivity to Zeitgebers and masking factors, are responsible for such differences in the timing of activity, and to what extent extrinsic factors, like exposure to artificial light at night, contribute to consistent behaviour. When studying inter-individual variation in the timing of activity, the spatial and temporal variation in the environment should be taken into account, as the relative contribution of intrinsic and extrinsic factors to among individual differences is of the utmost importance to understand the evolutionary potential of chronotypes (Niemelä & Dingemanse, 2017; Zsebők et al., 2017).

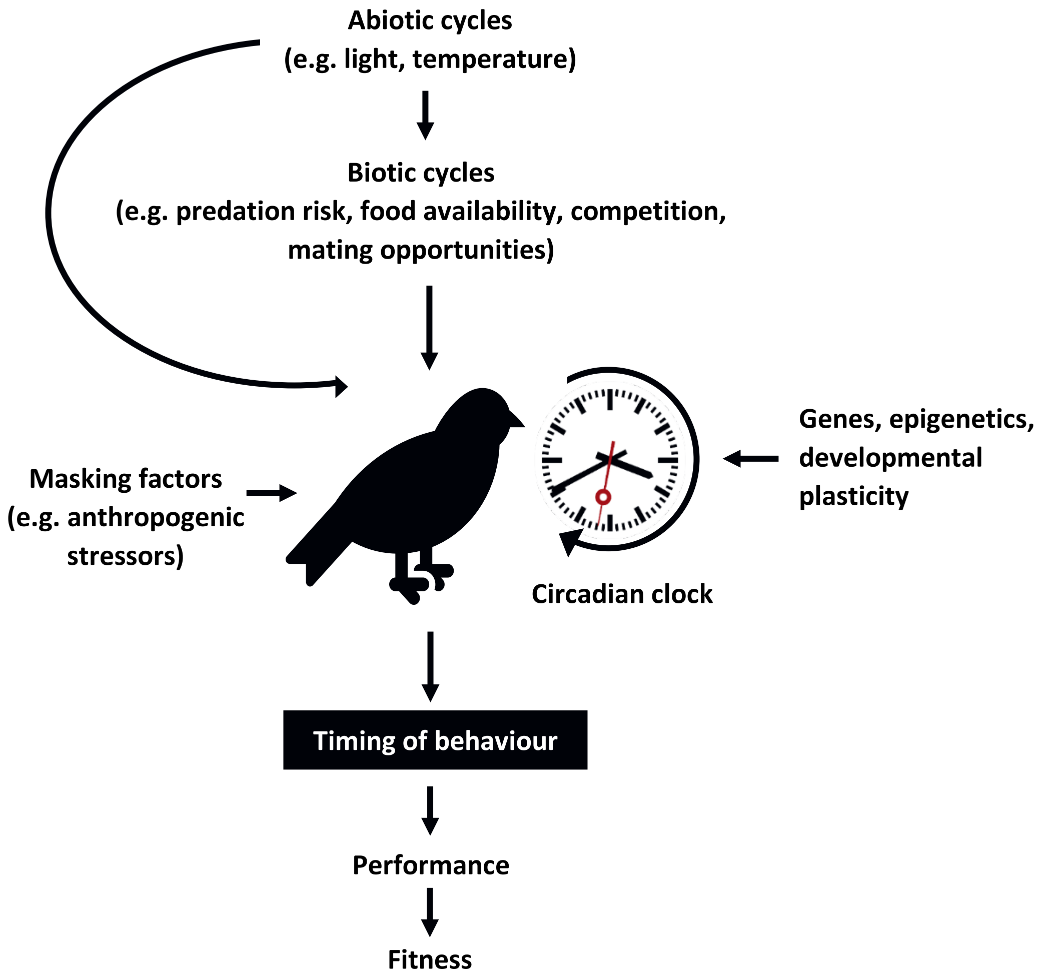

Figure 1: Schematic of the regulation of timing of activity in birds. An individual perceives abiotic and biotic cycles in the environment, which entrains the circadian clock. The properties of the circadian clock may be determined by genes, epigenetic modifications and developmental plasticity. Together these determine the timing of behaviour, but simultaneously masking factors perceived by the individual may modify the timing of behaviour. The timing of behaviour potentially affects an individual's performance, which may ultimately determine fitness. Based on (Helm et al., 2017).

Consequences for reproductive success and survival

In natural populations variation in behavioural traits is often present and such variation may come with consequences for reproductive success and survival. In my thesis I will focus on the consequences of the variation in the timing of activity, as in the wild, individuals are exposed to many challenges that require a correct timing of activity, which could ultimately affect fitness. In songbirds for example, predation risk may be highest during dawn and dusk, when both diurnal and nocturnal predators are active and vision is suboptimal (Krams, 2000). In addition, in temperate climates, temperature fluctuations may be reduced in amplitude inside a nest cavity or burrow, and resting during the coldest part of the day may thus decrease energy expenditure (Kendeigh, 1961; van der Veen et al., 2017; van der Vinne et al., 2015). Furthermore, food availability may depend on the circadian rhythm of prey (Daan & Aschoff, 1982), but also on competition with both conspecifics and heterospecifics (Alanärä et al., 2001; Gao et al., 2020; Krams, 2000; Kronfeld-Schor et al., 2017). On top of that, the timing of activity, such as the timing of the dawn chorus in birds, may serve as a quality signal during partner choice (Hau et al., 2017). Indeed, male songbirds that initiated the dawn chorus earlier had higher probabilities of gaining extra pair young and reduced the risk of being cuckolded in several bird species (Dolan et al., 2007; Greives et al., 2015; Poesel et al., 2006; Santema & Kempenaers, 2023; Schlicht et al., 2023; Steinmeyer et al., 2013).

It is likely impossible to maximize all opportunities while minimizing all risks simultaneously. However, there may potentially exist an optimal daily time window for being active (van der Veen et al., 2017), albeit limited plasticity in the timing of activity may hinder individuals from timing their activity optimally. Differences among individuals in their timing of activity may thus expose them to different risks and opportunities, which could have consequences for their reproductive success and survival. Moreover, recent studies in birds have shown that the timing of activity onset and offset differ between males and females (Schlicht & Kempenaers, 2020; Stuber, Dingemanse, et al., 2015; Stuber, Mathot, et al., 2015) and that the association between early activity and extra pair copulations as shown in males (see above), could not be confirmed in females (Schlicht et al., 2014; Steinmeyer et al., 2013). Together, these results indicate that the selective forces that shape the chronotype landscape may differ among the sexes and that the trait optima for timing of activity in males and females not necessarily coincide. It is thus essential to investigate the associations with reproductive success and survival in both males and females.

Differences in the optimal timing of activity between males and females could potentially lead to sexually antagonistic selection, a process that can maintain variation in a trait (Cox & Calsbeek, 2009). However, even within the sexes individuals may occupy different ecological niches, resulting in different trait optima based on their individual niche specialisation (Bolnick et al., 2003; Edelaar et al., 2008). Additionally, trait variation can be maintained through a combination of different processes, such as through spontaneous mutations or through disruptive selection (Brommer & Class, 2017). Frequency-dependent selection may also play a crucial role in maintaining variation in chronotypes, as the benefits of a specific timing can depend on the timing of other individuals (Wolf & McNamara, 2012). Furthermore, selection pressures may differ depending on the environment in which an individual lives, leading to spatially or temporally fluctuating selection pressures (Smith & Blumstein, 2008). Finally, correlations with other traits, such as the seasonal timing of reproduction or other behaviours, could also maintain the variation.

The role of the circadian rhythm in the seasonal timing of reproduction

Consequences of the circadian rhythm for fitness may also result from its involvement in the circannual rhythm of reproduction. Many species use photoperiod as a cue to entrain the annual cycle and the circadian clock can be used to measure its duration (Helm, 2020). Light perceived during a specific part of the day, i.e. the photo-inducible phase, triggers a photoperiodic reaction. In temperate-zone birds in spring, this photoperiodic reaction results in hormonal changes that affect the timing of reproduction. Differences among individuals in the circadian rhythm may thus result in differences in the daily timing of the photo-inducible phase, which ultimately affects the seasonal timing of clutch initiation (Graham et al., 2017; Helm & Visser, 2010; but see Liedvogel et al., 2009; Liedvogel & Sheldon, 2010). Indeed, in dark eyed juncos (*Junco hyemalis*) and great tits, a correlation between the timing of activity onset and the timing of reproduction (i.e. the laying date of the first egg) exists (Graham et al., 2017). Such a correlation can have important fitness consequences, since earlier timing of reproduction has been related to higher reproductive success (e.g. Christians et al., 2001; Garamszegi et al., 2004; Verboven & Visser, 1998; Verhulst et al., 1995). However, earlier activity onset may also result in longer active daylengths and consequently in more time available to collect the necessary resources to reproduce (Maury et al., 2020). The underlying mechanisms that cause a relationship between the timing of activity onset and the timing of seasonal reproduction have never been investigated. Nevertheless, an association between chronotype and the timing of reproduction may be key in maintaining variation in chronotypes, as selection pressures on the daily timing of activity may differ from selection pressures on the timing of reproduction, potentially resulting in trade-offs. Especially in the light of climate change and its associated pressure for earlier reproduction (Gienapp et al., 2013; Visser et al., 1998), understanding the relationship between the circadian and circannual rhythm is pivotal (Helm & Visser, 2010).

Behavioural syndromes

Furthermore, fitness consequences of chronotypes may not only depend on its mechanistic link with the timing of reproduction, but also on correlations with other traits. Different traits are often not independent from one another (Bell, 2006; Sih et al., 2004) and such suites of traits can be adaptive if the combination of traits is favoured by selection (Bell, 2005; Herczeg & Garamszegi, 2012). For example, risk-taking individuals may need to be fast explorers as they are more exposed to threats, whereas for risk-averse individuals being fast may come with too high energetic costs, since they may generally stay closer to cover and do not need to be fast. Such alternative strategies may be equally adaptive and both phenotypes may thus be maintained in the population. They likely depend on trade-offs between life-history characteristics, such as the investment in current and future reproduction (Dammhahn et al., 2018; Réale et al., 2010). If such correlations between traits involve two or more behaviours it is typically called a behavioural syndrome. However, behavioural syndromes are not necessarily adaptive and may even constrain evolution if they arise because of shared underlying drivers, such as pleiotropic genes or hormones (Dingemanse & Dochtermann, 2013; Dochtermann, 2011; Sih et al., 2004). Hence, the inability of the involved traits to evolve independently may result in traits that never reach their trait optima. Research on chronotypes within a behavioural syndrome framework is still in its infancy in non-human species and correlated behaviours that may have an impact on the evolutionary potential of chronotypes are therefore still largely undiscovered (but see Alós et al., 2017; Amin et al., 2016; Martorell-Barceló et al., 2023). However, correlations with other behaviours can be expected. For example in the context of intra-specific competition, which may drive less competitive individuals to the edges of the timing landscape, i.e. early in the morning or late in the evening (see Kronfeld-Schor et al., 2017; Martorell-Barceló et al., 2023). A behavioural syndrome between competitive ability and chronotype may thus be adaptive if less competitive individuals can improve their access to resources by being active when others are inactive. Such correlations likely have important ecological and evolutionary implications for the adaptive consequences of chronotypes and therefore deserve more attention.

Timing in a changing world

Chronotypes have evolved under natural conditions, but since human activity covers an ever increasing portion of the globe, many habitats are now polluted with anthropogenic stressors, which may increasingly impact the timing of activity in wild species. Indeed, significant differences have been observed between behavioural timing in city birds and rural populations. For example, blackbirds (*Turdus merula*) start activity earlier in the morning in urban sites compared to rural areas (Dominoni et al., 2014), and the timing of activity onset and offset in urban female great tits show different relationships with changes in daylength than those of rural females (Womack et al., 2023a). Especially artificial light at night (Da Silva et al., 2014; de Jong et al., 2017; Kempenaers et al., 2010; Raap et al., 2015; Raap, Pinxten, et al., 2016; Strauß et al., 2024) and noise pollution (Dorado-Correa et al., 2016; Fuller et al., 2007; M. L. Grunst et al., 2021) seem to interfere with the circadian rhythm, although they probably mainly work as masking factor, and not as Zeitgeber (Spoelstra et al., 2018). Despite increasing evidence that animals can adjust their activity timing in response to anthropogenic disturbance, it is largely unknown whether such changes in the timing of activity are adaptive, since associations with performance have hardly been studied (Gilbert et al., 2023). However, disruption of the sleep-wake rhythm by anthropogenic stressors may lead to mistimed behaviour (Gilbert et al., 2023) and sleep deprivation (Aulsebrook, Connelly, et al., 2020; Raap, Pinxten, et al., 2016) and may have severe consequences for wildlife. For example, body mass is reduced after acute exposure to artificial light at night (Raap, Casasole, et al., 2016; Strauß et al., 2024) and cognitive performance may be impaired (Aulsebrook et al., 2021). Such cognitive impairment could have direct consequences for survival. Therefore, there is an immediate need for research exploring the impacts of anthropogenic stressors on sleep deprivation and cognitive functioning in birds.

Study species and study population

To increase our understanding of the adaptive consequences of chronotypes and its responses to environmental change, it is pivotal to study wild individuals. An important model species in the study of avian circadian rhythms is the great tit, a passerine that is widely distributed across Europe and Asia (Song et al., 2020), which has been extensively used as study species in (behavioural) ecological and evolutionary research.

Naturally, they are cavity nesting (Gosler et al., 2020), but when nest boxes are available they readily accept them for roosting and breeding. This enables easy monitoring of reproductive success and allows measuring devices to be attached to the nest box for a wide variety of measurements, including measurements on the timing of activity (e.g. Grunst et al., 2021; Raap et al., 2015; Schlicht & Kempenaers, 2020). Great tits are largely monogamous, although extra pair copulations regularly occur (about eight percent of all nestlings is extra pair sired; Lubjuhn et al., 1999; Strohbach et al., 1998). Both pair members build a nest together in spring and after nest building is completed, the female lays one egg each morning before leaving the nest box on generally 5-12 consecutive days (Boyce & Perrins, 1987). However, egg laying is sometimes interrupted for a couple of days (Kluijver, 1951), which is related to cold temperatures (Lessells et al., 2002). Only the female is involved in incubation, which takes 12 to 14 days. Both pair members provision the young with food (Perrins, 1965). Great tits are non-migratory. During the breeding season, only the female sleeps inside the nest box, while in winter more males than females sleep inside the nest box. However, many individuals do not sleep inside nest boxes during this time of the year at all.

Great tits are predominantly insectivorous, heavily depending on caterpillars to raise their young (Perrins, 1991), but also feed on seeds, especially in winter (Vel’ký et al., 2011). Males are generally larger than females and are often dominant in feeding contexts (Krams, 2000). Furthermore, yearlings are often subordinate to older individuals. Other competitors can be the blue tit (*Cyanistes caeruleus*) and the pied flycatcher (*Ficedula hypoleuca*), which both feed on insects too and use the same nest boxes (Sasvári et al., 1987). The main predators for adult great tits in the studied population are sparrow hawks (*Accipiter nisus*), tawny owls (*Strix aluco*), corvids (*Corvidae*) and domestic cats *(Felis silvestris catus*; Curio et al., 1983; Pavisse et al., 2019; Sasvári & Hegyi, 1998).

In this thesis, free-living great tits were studied breeding in nest boxes in a suburban population in Antwerp, Belgium (51°09′46.1"N, 4°24′13.3"E). In this population nest boxes suitable for great tits are available since 1995 and the population has continuously been monitored for individual reproductive success since 1997 (Dauwe et al., 1999; Raap et al., 2015; Van Duyse et al., 2005). The population contains about 170 nest boxes and spans an area of 2.4 km^2. As this population is located in a suburban area, it is highly fragmented and very diverse in habitat type and the level of anthropogenic disturbance. For example, the Antwerp University Hospital and one of the University's campuses are located within the study area and the population is intersected by a highway. These infrastructures are interspersed with rural areas.

General research goals

The overarching goal of my thesis is to study the functional consequences of among individual variation in chronotypes in the great tit, while focusing on the daily timing of activity onset and offset. As circadian rhythms were studied in the wild in this thesis, proxies for the timing of activity had to be used. For the timing of activity onset the emergence time from the nest box in the morning was used and for the timing of activity offset the last entry time into the nest box in the evening was determined (e.g. Graham et al., 2017; Schlicht & Kempenaers, 2020; Womack et al., 2023a). To understand the consequences of the timing of activity for reproductive success and survival it is essential to first investigate whether individuals indeed show consistent differences in the timing of activity and how the timing of activity varies over temporal and spatial scales. In **Chapter 3**, I investigated changes in this timing across years using a long-term dataset. Great tits sometimes moved between nest boxes across years, which resulted in a dataset that included individual females that were each measured in more than one nest box as well as multiple females that were measured in the same nest boxes, allowing the estimation of the amount of variation in the timing of activity onset that was determined by the location. If individuals indeed show intrinsic differences in the timing of activity onset and offset, this could indicate that the trait is genetically underpinned. Such variation may be subjected to evolution when the variation is associated with fitness. Therefore, the consequences of the timing of activity onset and offset for reproductive success and survival were studied, and as the consequences may differ between the sexes, they were studied for both females (**Chapter 6**) and males (**Chapter 7**). These reproductive and survival consequences may be affected by the timing of reproduction, which could be mechanistically linked to the chronotype, so in **Chapter 2,** I tried to unravel the association between the timing of reproduction (i.e. clutch initiation date) and timing of activity onset. Furthermore, the fitness consequences of variation in the timing of activity may be affected by associations with other behaviours (i.e. behavioural syndromes), which were explored in **Chapter 5.** Specifically, I investigated the correlation between timing of activity onset and aggression as I hypothesized that less aggressive individuals may try to reduce competition with conspecifics by adjusting their activity timing to earlier hours, when social density is lower. **Chapter 4** deals with the potential consequences of global change, more specifically with the cognitive consequences of disruption of the sleep-wake cycle by artificial light at night. In an experimental set up, I exposed individuals to artificial light at night and compared their cognitive performance the next day with birds that were not exposed. Finally, in **Chapter 8**, all results will be synthesised and discussed. In this thesis, the chapters are presented in chronological order based on implementation.

Chapter 2

No relationship between chronotype and timing of breeding when variation in daily activity patterns across the breeding season is taken into account

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Published in Ecology and Evolution, 2022, 12, e9353

https://doi.org/10.1002/ece3.9353

Abstract

There is increasing evidence that individuals are consistent in the timing of their daily activities, and that individual variation in temporal behaviour is related to the timing of reproduction. However, it remains unclear whether observed patterns relate to the timing of the onset of activity or whether an early onset of activity extends the time that is available for foraging. This may then again facilitate reproduction. Furthermore, the timing of activity onset and offset may vary across the breeding season, which may complicate studying the above mentioned relationships. Here, we examined in a wild population of great tits (*Parus major*) whether an early clutch initiation date may be related to an early onset of activity and/or to longer active daylengths. We also investigated how these parameters are affected by the date of measurement. In order to test these hypotheses we measured emergence and entry time from/into the nest box as proxies for activity onset and offset in females during the egg laying phase. We then determined active daylength. Both emergence time and active daylength were related to clutch initiation date. However, a more detailed analysis showed that the timing of activities with respect to sunrise and sunset varied throughout the breeding season both within and among individuals. The observed positive relationships are hence potentially statistical artifacts. After methodologically correcting for this date effect, by using data from the pre-egg laying phase, where all individuals were measured on the same days, neither of the relationships remained significant. Taking methodological pitfalls and temporal variation into account may hence be crucial for understanding the significance of chronotypes.

Introduction

Circadian rhythms occur on a diel (24 h) time scale and are ubiquitous in all living organisms. They are endogenously orchestrated by the biological clock, but entrained by the light-dark cycle, so that they match the 24 hour daylength (Pittendrigh, 1993). However, the free-running period length (τ), which represents the amount of time it takes the endogenous clock to repeat itself in the absence of environmental cues, often differs slightly from 24 hours and it intriguingly varies among individuals too (Helm & Visser, 2010). This individual variation in the functioning of the biological clock becomes visible at the phenotypic level as consistent among-individual variation in the timing of activities. The early or late timing of events is referred to as ‘chronotype’. It typically captures the timing when an individual starts with its activity in the morning and when it becomes inactive in the evening. In humans variation in the preferred timing of activities is referred to as ‘morningness’ and ‘eveningness’ (Arrona-Palacios et al., 2020). Variation in the timing of activity patterns have been found in a variety of other taxa, including mammals and birds, both in laboratory settings and free-living populations (e.g. Labyak et al., 1997; Lehmann et al., 2012; Refinetti et al., 2016; Steinmeyer et al., 2010). Thus, it is commonly accepted that individuals consistently differ from each other in the timing of their activity patterns.

Understanding how this individual variation in chronotypes is maintained in natural populations is of outermost relevance, but knowledge about the evolution and adaptive significance of chronotypes in natural ecosystems is still scarce (Dominoni et al., 2017; Helm et al., 2017). However, recently there is an increased interest in this topic. Furthermore, while existing studies are often laboratory-based, where testing functional consequences or even fitness consequences is difficult (van der Veen et al., 2017), studies on chronotypes are now taken into the wild. Here, it can be expected that chronotypes are under both sexual and natural selection, as chronotypes may influence the timing of the expression of certain traits (Hau et al., 2017). For example, dawn song in male birds should be timed precisely to the presence of (receptive) females (Hau et al., 2017), while timing might also play an important role for minimising predation risk and maximising foraging efficiency (DeCoursey et al., 2000; Helm et al., 2017).

Still, empirical evidence on the fitness consequences of chronotypes is mixed. Both male and female birds that engaged in extra pair copulations, which particularly occur at dawn, had earlier chronotypes than other birds (Halfwerk et al., 2011; Poesel et al., 2006), but this could not be confirmed in a later study (Schlicht et al., 2014). Maury et al. (2020) and Steinmeyer et al. (2013) found that clutch size and number of fledglings were independent from temporal phenotype in females, but Graham et al. (2017) reported that females which had an earlier onset of activity in the morning had earlier clutch initiation dates. The latter is commonly assumed to be a fitness measure, as earlier hatched chicks have higher recruitment rates (e.g. Verboven & Visser, 1998). This suggests that the timing of reproduction rather than the reproductive investment might vary with chronotype.

However, if early rising females have a similar timing for the offset of activity as late rising females, this would lengthen their active day (i.e. the time they spend outside the nest box) and the time they can, for example, spend on foraging. Early rising, and thus increasing active daylength, would then allow individuals to make more efficiently use of the limited resources at the beginning of the breeding season, as they would have more time available. The active daylength can be further increased by delaying the cessation time, as has recently been reported for female European starlings (*Sturnus vulgaris*), where individuals with an early onset of activity had later cessation times than females which had a late onset of activity (Maury et al., 2020). Also in blue tits, substantial variation among individuals has been shown for active daylength, so that a distinction between long and short sleeping individuals could be made (Steinmeyer et al., 2010). This altogether implies that a relationship between activity onset in the morning and clutch initiation date may not only depend on the timing of daily activity but could also be the result of an increase in active daylength in early rising individuals.

Furthermore, a concern that has potentially not sufficiently been taken into account in previous studies on the fitness consequences of the daily timing of activity is the contribution of temporal variation across the breeding season as underlying driver of such relationships between fitness and timing of activity. Emergence time, entry time and therewith active daylength, which are key parameters when studying individual variation in temporal behaviour, vary throughout the year (Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015), even after correcting for the seasonal changes in the timing of sunrise and sunset. This suggests that the significance of sunrise and sunset for determining activity patterns may differ across the year or with date of measurement both within and among individuals. The date of measurement may thus be a confounding factor when analysing relationships between the activity parameters and fitness estimates such as clutch initiation date, which are temporal parameters in itself.

Here, we study the relationships between activity patterns at the onset of reproduction and clutch initiation date (Graham et al., 2017), as measured by regular nest checks in a nest box breeding population of great tits. First, we investigate whether individual variation in activity patterns is consistent (i.e. repeatable) within and across periods (pre-egg laying and egg laying) in the breeding cycle. Then, we investigate whether the daily timing of onset of activity in the morning is related to the seasonal timing of onset of reproduction, i.e. start of egg laying. By considering both onset (here: emergence time from the nest box) and offset (here: nest box entry time in the evening) of daily activity, we also investigate the hypothesis that earlier rising females have longer active daylengths (i.e. advanced onset but not advanced offset), which allows them to accumulate the relevant resources earlier in the breeding season, so that they can start reproduction earlier in the season. Finally, we investigate whether the above described relationships may be affected by variation in the daily timing of activity across the breeding season.

Material and Methods

Population

This study was carried out in a suburban nest box population of great tits, located in Wilrijk (Antwerp), Belgium (51°09'46.1"N, 4°24'13.3"E) during the breeding season

(March – June) of 2020 (Raap, Pinxten, et al., 2016; Rivera-Gutierrez et al., 2012; Van Duyse et al., 2005). About 170 nest boxes, suitable for great tits, are placed in trees at a height of about two meters. All individuals that had been captured during previous breeding seasons or during roosting in winter were equipped with a ring containing a PIT-tag (passive integrated transponder; EM4102, 125 KHz, Eccel Technology Ltd, Aylesbury, UK) and a unique combination of colour rings, enabling individual recognition. The nest boxes were checked every few days for nest building, egg laying and incubation. In our population great tits can have up to two broods per year, but this study only contains data of first breeding attempts.

Emergence and entry times

In order to determine the time at which females leave the nest box in the morning (emergence time) and enter in the evening (entry time) we used SongMeters (SongMeterTM SM2+; Wildlife Acoustics, Inc, U.S.) and radio-frequency identification (RFID) loggers (EM4102 data logger, Eccel Technology Ltd, Aylesbury, U.K.). RFID loggers consist of two antennas, which were placed around the opening of the nest box, one on the inside, the other on the outside. When a PIT-tagged individual passes through the antennas, the RFID logger registers the unique PIT-tag number and the time of passing (for more details see Iserbyt et al., 2018). The reader sample interval was set to 250 ms and the sleep mode between 10:00 p.m. and 03:00 a.m. As not all individuals in the population were equipped with PIT-tags, we also used SongMeters to determine emergence and entry times. SongMeters have two microphones to record sounds both inside and outside the nest box. Both microphones produce sonograms. Before the clock changed to summer time, sound was recorded in the morning from 04:00 a.m. to 08:00 a.m. CET and in the evening from 05:30 p.m. to 08:30 p.m. CET. After the clock changed to summertime we recorded sound from 03:00 a.m. to 08:00 a.m. CET in the morning and in the evening from 05:30 p.m. to 09:00 p.m. CET. Morning emergence time and evening entry time could be determined by the sound of the female's claws on the nest box (microphone inside) and the sound of her wings when taking off (microphone inside & outside) (Halfwerk et al., 2011). Furthermore, a specific sound caused by a change in air pressure can be heard when the female passes the opening of the nest box. Data recorded by SongMeters were analysed using Avisoft SASLab Pro 5.2.14 (Specht, 2002).

Emergence and entry times were measured during the egg laying phase (i.e. after the first egg was laid and before incubation started) and for a subset of individuals also during the pre-egg laying phase (i.e. when nest building was completed and before the first egg was laid; see below). As individuals shift the timing of activity substantially between the different stages of breeding (Schlicht & Kempenaers, 2020), the physiological state should not differ between individuals when measuring activity patterns. During the pre-egg laying phase all individuals should thus be measured once nest building is completed. However, not all females sleep in the nest box during this phase, and many females finish nest building only the day before egg laying starts. This does not allow obtaining large sample sizes during the pre-egg laying phase. During the egg laying phase however, all females sleep in the nest box and measuring all individuals in the same physiological state is relatively easy. As timing of activity is thought to be consistent we expected that individuals with relatively early timing during the egg laying phase would also be early during the pre-egg laying phase. We showed that emergence time is repeatable on the long term (i.e. across years) in female great tits in our population (Meijdam, Müller, & Eens, 2022). Therefore, we decided to measure emergence and entry times mainly during the egg laying phase.

We used a combination of both SongMeters and RFID loggers. Emergence times were measured 88 times with both SongMeter and RFID logger. Twenty-seven percent of the measurements by RFID loggers did not correspond with the SongMeter. Visual validation of our RFID loggers was performed in previous years both in blue tits and great tits. In blue tits in a dataset of 242 parental visits (N = 10 nests) 86.8% of all entries and 43.8% of all departures were registered (Iserbyt et al., 2018). In great tits the correlation between feeding rates of females measured with RFID loggers and cameras was 0.78 (Thys et al., 2021; note: when feeding chicks females both enter and depart from the nest box so there are 2 chances to be registered). Thus, even though the speed when passing the RFID logger is much higher during chick rearing when compared to leaving the box after awakening, and is also faster in blue tits, there is still a chance that the entry or emergence time into/from the nest box will be missed by our RFID loggers. In almost all instances in which the SongMeter data did not correspond to the RFID logger data, the RFID logger showed later emergence times and earlier entry times than the SongMeter. Therefore, SongMeter data are likely more accurate and it is highly likely that the RFID loggers missed the first emergence and last entry from/into the nest box. Unfortunately, we do not have data to visually validate the data collected by SongMeters. However, determining emergence and entry times using SongMeters is straight forward (see Figure S1) and has successfully been used in previous papers (e.g. Halfwerk et al., 2011).

For these reasons, we decided to use only data from SongMeters if both SongMeter and RFID logger data were available. If only RFID logger data were available ($n_{observations}$ = 58 on 30 females), only measurements that fell within the range of emergence times measured by the SongMeters were included in the dataset (127 minutes before sunrise up to 63 minutes after sunrise; this resulted in the removal of 16 datapoints). For entry times the error was 12% on 82 measurements, so here we used the same procedure as for emergence times (an overview of the sample sizes after the data removal criterium was applied is presented in Table 1. A comprehensive overview of the number of birds sampled per day is presented in supplementary Table S1). Completely excluding the RFID data from the analyses did not change the outcome or interpretation (these results will not be further discussed).

Table 1: Sample sizes of emergence time, entry time and active daylength during the pre-egg laying phase and the egg laying phase.

Phase	Variable	Number of females	Number of measurements							
			Mean per female	Repeats per female						
				1	2	3	4	5	6	7
Pre-egg laying	Emergence time	23	2.96		1	22	0			
	Entry time	24	3.04		3	17	4			
	Active daylength	22	2.95		1	21	0			
Egg laying	Emergence time	121	3.84	1	5	49	27	36	2	1
	Entry time	116	3.54	3	23	30	35	19	5	1
	Active daylength	114	2.98	2	45	26	36	4	1	0

For both SongMeter and RFID data we determined emergence times relative to sunrise (negative = before sunrise, positive = after sunrise) and entry times relative to sunset (negative = before sunset, positive = after sunset). We also determined the relative active daylength (negative = shorter active period than the period between sunrise and sunset, positive = longer active period than the period between sunrise and sunset). Hereafter, emergence time, entry time and active daylength always concern relative times, unless it is specifically made clear that they concern absolute times. Temperature data was retrieved via: https://www.wunderground.com/history/daily/be/antwerp.

In models containing emergence time we used the temperature (T°) at sunrise, in models containing entry time we used T° at sunset and in models containing active daylength we used the maximum daily T° on the day of measurement.

Statistical analysis

All statistical analysis were performed in R 4.0.2 (R Core Team, 2013). We used the ‘rptR’ package (Stoffel et al., 2017) to calculate repeatabilities, which uses parametric bootstrapping to quantify confidence intervals, and likelihood ratio testing to determine statistical significance. Statistical significance of fixed effects for each linear mixed model was obtained with stepwise backwards elimination using lmerTest (Kuznetsova et al., 2017). For all statistical tests the significance level was set at α = 0.05.

In order to test if an individual’s average entry time depended on its average emergence time, both measured during the egg laying phase, a linear model was used. Second, we used two separate linear models to test whether clutch initiation date depended on the individual’s average emergence time or its average active daylength. Clutch initiation dates ranged from March 22 up to April 20 (= 30 days). In both models female age (in years) was included as fixed effect.

Although we used relative values for emergence and entry time in order to account for changes in the onset of dawn and dusk across the breeding season, a visual inspection of the data revealed that there could still be temporal variation in both parameters. To explore these patterns, we modelled variation in activity parameters in relation to the date of measurement, using random regression analyses (Dingemanse, Kazem, et al., 2010; Nussey et al., 2007). Three identical models were run for emergence time, entry time and active daylength. The models included the average date (starting as a count from the 1st of April) on which an individual was measured (= among-individual effect), the deviation from the average date (= within-individual effect) (van de Pol & Wright, 2009), their interaction and age of the female as fixed effects. The among-individual effect allows to test whether females, on average (population-level), differ in activity patterns when observed on different dates. The within-individual effect allows to test whether females, on the population level, plastically adjust their activity as the date progresses. The interaction allows to test whether plasticity depends on mean date of testing. As temperature is known to affect activity patterns in great tits (Lehmann et al., 2012; Stuber, Dingemanse, et al., 2015), we also included the temperature as described above. Random intercepts (= chronotype; i.e. do individuals differ from each other in average activity patterns?) were included for female ID and random slopes on the level of the deviation from the average date (= individual plasticity in activity patterns in response to date; i.e. do individuals differ from each other in plasticity?) were included for female ID as well. Stepwise backwards elimination of non-significant terms was performed to obtain the minimum adequate model (MAM). Likelihood ratio tests were used to determine significance of random effects (i.e. individual intercept and slope). Adjusted repeatabilities for emergence time, entry time and active daylength during the egg laying phase were calculated from these MAMs as the variance explained by female ID relative to the total variance.

As we suspected that the variation in emergence time, entry time and active daylength across the breeding season may have confounded the relationships between clutch initiation date and emergence time/active daylength, we decided to use additional data that we had collected during the pre-egg laying phase. During this phase we placed SongMeters on 25 nest boxes with nests that were completed, but with no eggs yet. For these 25 females, we measured emergence and entry times between March 26 and March 30. Temporal variation was thus very limited. We used linear mixed models to test whether emergence time, entry time and active daylength were affected by the date of measurement, the number of days prior to clutch initiation, the temperature, and the female's age (in years since birth, with age = 0 is year of birth; for the results see supplementary Figure S2 and supplementary Table S2). In all models, random intercepts were included for female identity (ID). After excluding non-significant fixed effects we calculated the adjusted repeatability.

We performed similar analyses as before to determine relationships between emergence time/active daylength measured during the pre-egg laying phase and clutch initiation date. Here, clutch initiation dates ranged from March 29 up to April 15 (= 18 days).

Additionally, using a separate model on the subset of females measured during both the pre-egg laying and the egg laying phase, we estimated the between-period repeatability of emergence time, entry time and active daylength ($n_{\text{emergence time}}$ = 23, $n_{\text{entry time}}$ = 24, $n_{\text{active daylength}}$ = 21). We included the reproductive phase as a two-level factor (i.e. pre-egg laying vs. egg laying) and the measurement interval (i.e. the number of days between the average measurement date during the pre-egg laying phase and the egg laying phase; mean = 8.3, min. = 3, max = 18.5) as a continuous covariate. Non-significant fixed effects were removed from the models. Both female ID and the unique combination of period and female ID were included as random effects, thereby specifically allowing to estimate the between-period repeatability, following (Araya-Ajoy et al., 2015). That is, the adjusted between-period repeatability was calculated from this model as the variance explained by the individual relative to the total variance.

Ethical note

This study was approved by the ethical committee of the University of Antwerp (ID numbers: 2016-87 and 2018-50) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complies with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licenses for all authors and technicians. Handling time was minimised as much as possible. All other methods described above are non-invasive.

Results

During the pre-egg laying phase emergence times ranged between 76 minutes before sunrise and 21 minutes after sunrise (Table 2). Entry times ranged from 63 minutes before sunset up to 10 minutes after sunset and active daylengths from 56 minutes shorter than the daylight period up to 46 minutes longer. During the egg laying phase emergence times ranged from 127 minutes before sunrise up to 63 minutes after sunrise. Entry times ranged between 136 minutes before sunset and 13 minutes after sunset. The shortest active daylength we measured was 153 minutes shorter than the daylight period and the longest active day was 35 minutes longer than the daylight period.

Table 2: Summary of the measured values for emergence time, entry time and active daylength (in minutes relative to sunrise, sunset and the period between sunrise and sunset respectively) during the pre-egg laying and egg laying phase.

Phase	Variable	Min.	Max.	Mean	SD
Pre-egg laying	Emergence time	-76	21	-17.51	13.64
	Entry time	-63	10	-23.07	17.25
	Active daylength	-56	46	-6.25	21.92
Egg laying	Emergence time	-127	63	5.81	17.59
	Entry time	-136	13	-39.18	24.67
	Active daylength	-153	35	-45.35	32.51

Repeatability of daily activity patterns

Both during the pre-egg laying phase and the egg laying phase the adjusted repeatability was significant for emergence time, entry time and active daylength (Table 3). In contrast, between-period repeatabilities for emergence time (R [95% CI] = 0.09 [0, 0.30]), entry time (R = 0.20 [0, 0.42]) and active daylength were not significant (R = 0 [0, 0]).

Table 3: Adjusted repeatability for emergence time, entry time and active daylength (in minutes relative to sunrise, sunset and the period between sunrise and sunset respectively) during the pre-egg laying and egg laying phase. All repeatabilities were calculated based on the MAM for the respective period and variable (for information on significant fixed effects see supplementary Table S2 and Table 4). 95% confidence intervals are shown between brackets. Estimates in bold are statistically significant (P < 0.05).

Phase	Variable	Adjusted repeatability
Pre-egg laying	Emergence time	**0.39 [0.10, 0.62]**
	Entry time	**0.27 [0.018, 0.52]**
	Active daylength	**0.45 [0.17, 0.67]**
Egg laying	Emergence time	**0.54 [0.43, 0.63]**
	Entry time	**0.77 [0.71, 0.83]**
	Active daylength	**0.71 [0.63, 0.80]**

Clutch initiation date and daily activity patterns during the egg laying phase

Females with an earlier emergence time during the egg laying phase ended their activities outside the nest box later during the day than females that showed a later onset of activity (t = -2.62, df = 112, P < 0.01). Emergence time was positively related to clutch initiation date (t = 3.85, df = 118, P < 0.001; Figure 1a). Individuals that started their activity early during the day laid their first egg earlier during the breeding season than individuals with late emergence times. In addition, active daylength was negatively related to clutch initiation date (t = -6.96, df = 111, P < 0.001; Figure 1b). Individuals that were longer active during the day laid their first egg earlier during the breeding season than individuals that were active for a shorter time period.

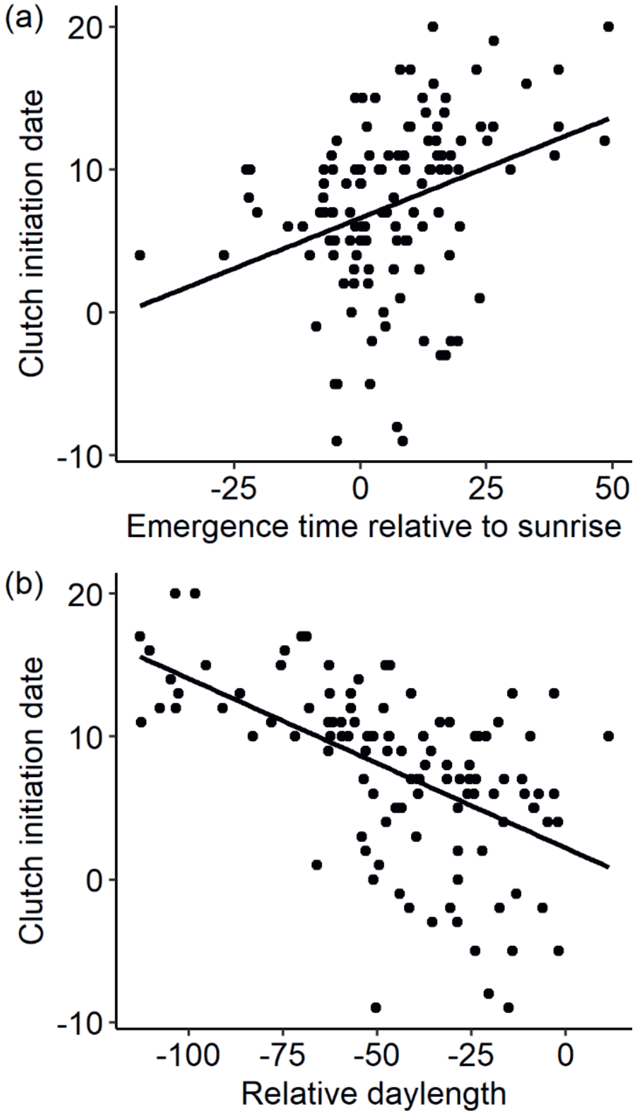

Figure 1: Average emergence times in minutes relative to sunrise (negative value = before sunrise) (a) and average active daylength in minutes relative to the period between sunrise and sunset (negative value = shorter active than the period between sunrise and sunset) (b) as measured during the egg laying phase both affected the clutch initiation date (starts as a count from April 1 (= 1)).

The influence of date on daily activity patterns

Date had an important influence on emergence time, entry time and active daylength (Figure 2; Table 4). Early in the season individuals emerged close to sunrise (= 0), while later in the season emergence times became later (= positive values; Figure 2a). Thus, date had a positive effect on emergence times (Average date effect is given in Table 4). This effect was partly driven by an among-individual effect, but at the same time, emergence time became later on consecutive days within individuals (Date deviation effect in MAM: $t = 3.23$, $df = 296.72$, $P < 0.01$; Figure 2a). Furthermore, older females had earlier emergence times (Table 4). Temperature at sunrise did not affect emergence times. On average, entry time became earlier as the date progressed (Figure 2b) and active daylength shorter (Figure 2c; Table 4). Within individuals these effects on entry time and active daylength became in both cases stronger towards the end of the breeding season, as indicated by the significant interaction between average date and date deviation (Table 4). Age did not have an effect on either entry time or active daylength. Active daylength was longer on warmer days (Table 4), but temperature at sunset did not affect entry time.

Table 4: Results from linear mixed effects models with random intercepts and slopes for testing the influence of date on emergence time, entry time and active daylength (in minutes relative to sunrise, sunset and the period between sunrise and sunset respectively) during the egg laying phase. Estimates in bold are statistically significant (P < 0.05).

Dependent variable	*Fixed effects*	β	SE	t	df	P
Emergence time	Average date	**0.61**	**0.21**	**2.94**	**106.72**	**<0.01**
	Date deviation	1.25	1.31	0.95	80.28	0.34
	Age	**-2.98**	**1.29**	**-2.31**	**92.37**	**0.02**
	$T_{sunrise}$	-0.31	0.25	-1.25	350.26	0.21
	Average date x date deviation	0.11	0.12	0.95	76.49	0.56
	Random effects	σ²		χ²	df	P
	$ID_{intercept}$	**118.07**		**119.34**	**1**	**<0.001**
	ID_{slope}	14.33		3.79	2	0.15
	$Corr_{intercepts-slopes}$	-0.03				
	Residual	93.14				
	Fixed effects	β	SE	t	df	P
Entry time	Average date	**-1.96**	**0.27**	**-7.19**	**99.32**	**<0.001**
	Date deviation	0.66	2.45	0.27	111.04	0.79
	Age	-1.79	1.62	-1.11	87.96	0.27
	T_{sunset}	0.14	0.17	0.81	293.66	0.42
	Average date x date deviation	**-0.94**	**0.21**	**-4.48**	**99.28**	**<0.001**
	Random effects	σ²		χ²	df	P
	$ID_{intercept}$	**212.57**		**114.89**	**1**	**<0.001**
	ID_{slope}	**83.25**		**50.76**	**2**	**<0.001**
	$Corr_{intercepts-slopes}$	0.32				
	Residual	87.23				

	Fixed effects	β	SE	t	df	P
Active daylength	Average date	**-2.71**	**0.34**	**-7.94**	**105.84**	**<0.001**
	Date deviation	-0.54	3.07	-0.18	75.59	0.86
	Age	0.34	2.11	0.16	87.68	0.87
	Tmax	**0.76**	**0.26**	**2.91**	**262.75**	**<0.01**
	Average date x date deviation	**-1.05**	**0.27**	**-3.87**	**73.51**	**<0.001**
	Random effects	σ^2		χ^2	df	P
	$ID_{intercept}$	**304.74**		**92.30**	**1**	**<0.001**
	ID_{slope}	**83.97**		**6.83**	**2**	**0.03**
	$Corr_{intercepts\text{-}slopes}$	0.01				
	Residual	184.06				

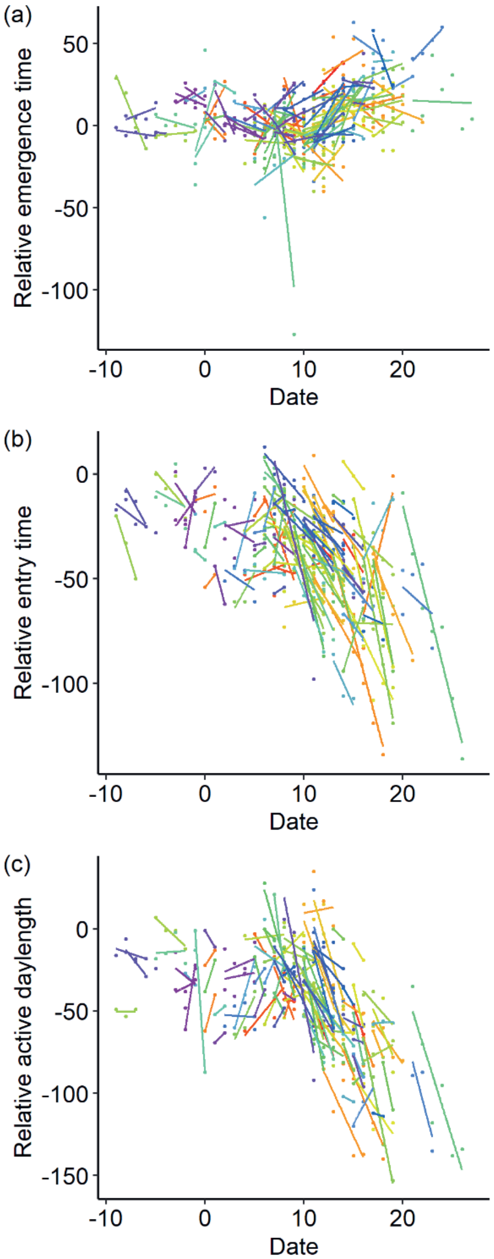

Figure 2: Activity patterns in female great tits are dependent on the date: a) emergence times relative to sunrise, b) entry times relative to sunset and c) active daylength in minutes relative to the period between sunrise and sunset. All individuals have separate regression lines (individuals can be distinguished by colour). Date starts as a count from April 1 (= 1).

Clutch initiation date and activity patterns during the pre-egg laying phase

During the pre-egg laying phase entry times were not related to emergence times (a subset of females, n = 23, t = -0.44, df = 22, P = 0.66). Furthermore, during the pre-egg laying phase, neither emergence time (t = -0.25, df = 3,18, P = 0.81), nor active daylength (t = 1.58, df = 2,19, P = 0.13) were related to clutch initiation date.

Discussion

Our initial analysis supported the previously reported finding that female great tits with an early onset of activity start to reproduce earlier in the season (Graham et al., 2017). The data equally supported our hypothesis that the relationship between clutch initiation date and emergence time is driven by active daylength, i.e. the time a female has available for foraging. However, when taking the date effect on the activity measures into account, by using data from the pre-egg laying phase (where all individuals were measured on the same days), neither of these relationships remained significant. The consequences thereof for this and previous studies will be discussed below.

Repeatability

During the egg laying phase repeatability of all activity measures was high, which suggests the existence of chronotypes in female great tits (Lehmann et al., 2012; Maury et al., 2020; Schlicht & Kempenaers, 2020). Also during the pre-egg laying phase activity measures were moderately and significantly repeatable. Contrary to our expectation, the between-period repeatability (i.e. across the pre-egg laying and egg laying phase) of emergence time, entry time and active daylength was non-significant. When studying the relationship between daily timing of activity and clutch initiation date, the timing of activity should thus preferentially be measured before egg laying starts. However, in our initial analyses and in previous studies on this relationship the timing of activity was measured during later periods (Here: egg laying phase, Graham et al., 2017: incubation, Maury et al., 2020: incubation, Helm & Visser, 2010: autumn). The lack of repeatability between the different periods may be due to the small sample sizes during the pre-egg laying phase. Especially for longer term repeatability small sample sizes can cause great imprecision in the estimate for among individual variation and low power to detect significance, which affects the repeatability estimate (Araya-Ajoy et al., 2015). Therefore, it will be of interest to investigate long-term (i.e. cross-season and cross-year) repeatability of activity patterns in more detail and with larger sample sizes in the future.

Emergence times versus active daylength

Initially, using the data from the egg laying phase, we found a positive relationship between emergence time and clutch initiation date, which is in accordance with results of a recent study on great tits and dark eyed junco's (*Junco hyemalis*, Graham et al., 2017). However, this relationship had not been found in captive great tits (Helm & Visser, 2010), in free-living European starlings (Maury et al., 2020) and in free-living blue tits (*Cyanistes caeruleus*, here: awakening time was used, which is highly correlated with emergence time, Steinmeyer et al., 2013). One possible explanation for the discrepancy between these studies might be that environmental factors that could affect the relationship may vary from year to year. For example, spring temperature may modulate the effect of light as trigger for the onset of breeding (Dominoni et al., 2020). Studying the relationship between chronotype and clutch initiation date in multiple years may reveal the impact of such environmental variation. As we hypothesised above, another possibility could be that active daylength rather than emergence time plays a role in determining onset of egg laying. Our initial analyses indeed show that individuals with longer active daylengths initiated egg laying earlier in the season and that individuals that emerged earlier from the nest box entered it later compared to late rising individuals (Maury et al., 2020; Steinmeyer et al., 2010; but Stuber, Dingemanse, et al., 2015 only showed this effect within individuals).

However, emergence time, entry time and active daylength were measured during egg laying and, as a consequence, they were measured soon after clutch initiation (i.e. most often measurements started 1 or 2 days after clutch initiation). The date of measurement was thus very tightly linked to the laying date of the first egg and differences in activity patterns between early and late laying females could possibly be explained by environmental changes over time (e.g. temperature, food availability, predation risk and light intensity at the nest box due to an increase in leaf coverage) instead of intrinsic differences in chronotypes. Therefore, we expected that the date of measurement may be a confounding factor when analysing the relationships between the activity parameters and clutch initiation date.

To tackle this problem, and because we did not find repeatability in the daily timing of activity between the different periods, we performed additional analyses that supported our presumption that the date of measurement is a confounding factor in the relationship between activity patterns and clutch initiation date. First, we tried to statistically correct for date of measurement, by using individual intercepts and slopes. However, it is not possible to disentangle date of measurement from clutch initiation date with this method. Instead, we used emergence times and active daylengths from a subset of females, that were measured during the pre-egg laying phase. All females were measured multiple times within a range of 5 days (i.e. the date of measurement was independent from the clutch initiation date). We found that neither emergence time nor active daylength measured during the pre-egg laying phase were related to the initiation of egg laying. Thus, as emergence times and active daylengths were not related to clutch initiation date when methodologically corrected for the date of measurement, we consider it most likely that the relationships we initially found are confounded by the date of measurement.

Variation in emergence time and active daylength across the breeding season

The date on which an individual was measured affected its emergence time, entry time and active daylength relative to sunrise and sunset, i.e. even after correcting for changes in sunrise and sunset over time. Emergence time delayed with date, while entry time advanced. A similar effect was recently reported in individual blue tits during the egg laying phase, but date effects on the population level were not investigated (Schlicht & Kempenaers, 2020).

As circadian clocks are entrained by the light-dark cycle (e.g. Berson et al., 2002; Wright et al., 2013; Zeng et al., 1996), light intensity is likely a very important determinant for activity patterns in the wild (see also Sockman & Hurlbert, 2020 for a discussion on the role of active daylength on migratory behaviour). In great tits and blue tits, light intensity at the nest box significantly influenced emergence time and awakening time in the morning, respectively (Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015). However, the variation that we observed in emergence and entry time relative to sunrise and sunset over time both within and among individuals, indicates that the light intensities that trigger emergence from and entry into the nest box change over time.

At present we can only speculate about the underlying drivers. During winter, when the days are short, individuals may have to make use of the full daylight period, while in spring, when the days are much longer, they may not need the full daylight period to perform all necessary tasks. Conversely, great tits may also need a minimal amount of sleep. Therefore, emergence times may delay relative to sunrise when the days lengthen while entry times advance. However, during the breeding season, we would then expect the absolute active daylength to remain constant from a certain moment onwards, but in fact it started to decrease while the daylight period was still lengthening.

In addition to light intensity alternative Zeitgebers (i.e. environmental factors that can entrain the biological clock) and masking factors (i.e. factors that do not change the internal clock time, but instead modify the expression of behavioural rhythms) may be important (Helm et al., 2017). For example, earlier studies showed that wild great tits delayed entry times on warmer evenings (Stuber, Dingemanse, et al., 2015), while captive great tits had later activity onset and earlier activity offset in warmer conditions (Lehmann et al., 2012). We found that an increase in maximum temperature was related to longer active daylengths, although temperature at sunrise and sunset did not significantly influence emergence and entry times. Temperature may thus modulate activity patterns, but it could not fully explain the changes over time as observed in our population.

Another environmental factor that could affect emergence and entry time is the food availability (Hau & Gwinner, 1997; Rani et al., 2009; Vivanco et al., 2010). When food is not continuously available, but only during specific time frames, this can entrain the biological clock and individuals may shift their circadian phase, in order to meet the requirements of optimal foraging. Alternatively, food availability may have acted as a masking factor. For example, on days with high food availability great tits may need less time for foraging in order to meet their energy requirements, which enables earlier cessation of activity in the evening (Bach et al., 2017; Northeast et al., 2020, but see Inoue et al., 2016). However, as we do not have data on food availability, the influence of environmental factors like food availability on emergence and entry times needs further investigation.

Furthermore, the amount of time spent on night time incubation may have affected activity patterns during egg laying. During this phase females already start incubating the eggs at night. With each subsequent egg the amount of time spent on night time incubation increases (Lord et al., 2011; Podlas & Richner, 2013) and females with late egg laying dates incubate longer at night than females with early laying dates (Haftorn, 1981). Night time incubation normally starts immediately after entering the nest box, but whether entry times advance when night time incubation increases is yet unknown.

Yet, none of the above mentioned factors seems to fully explain the observed temporal patterns in emergence and entry times.

As pointed out above the significance of sunrise and sunset for determining activity patterns changes over time both within and among individuals. This is relevant for interpreting this and previous studies, even though most of the previous studies did not find date effects on emergence times during the breeding season (Graham et al., 2017; Maury et al., 2020; Womack, 2020). This discrepancy may be caused by our much larger sample size and a larger range of dates on which we measured emergence times. Therefore, it is possible that, although Graham et al. (2017) did not find date effects on emergence times, their results may still be confounded by date. They measured emergence times during the incubation period, which is slightly different from our approach. However, if emergence time is measured at a fixed time after clutch completion, it could still be possible that date inflates the relationship between clutch initiation date and emergence time. In fact, Steinmeyer et al. (2013), who recorded sleep behaviour during multiple months in winter in multiple years, found that sleep parameters (including awakening time) varied greatly between recording dates and therefore they corrected awakening times for the date of measurement. The corrected awakening times then again did not affect clutch initiation dates.

Conclusions

We showed that both emergence time and active daylength (measured during the egg laying phase) were related to clutch initiation date, but both relationships were confounded by date of measurement, as the timing of measuring activity patterns was tightly coupled to the initiation of egg laying. When using methodologically corrected data from the pre-egg laying phase, we did not find a significant relationship between timing of activity and clutch initiation date. Furthermore, our results showed that the relevance of sunrise and sunset for the timing of activities varies throughout the breeding season, possibly in response to environmental factors such as temperature or food availability. This makes it methodologically extremely challenging to correct for date of measurement effects. Future studies on functional consequences of activity patterns should hence aim to vary the time span between the dependent (here: laying date) and independent (here: timing of activity) variable, e.g. by measuring activity patterns of all individuals on the same day(s), while being in the same breeding phase. Such confounding factors are possibly very common in statistical analyses including date. In addition, if individuals respond plastically to temporal changes in the environment, spatial differences in the environment may also affect activity patterns and could be partially responsible for differences in emergence times, entry times and active daylengths among individuals, which as yet needs to be investigated.

Supplementary material

The supplementary information accompanying this chapter can be found on pages 174 - 177 of this thesis and is available online at DOI:10.1002/ece3.9353.

Author Contributions

Marjolein Meijdam: Conceptualization (equal); formal analysis(equal); investigation (equal); methodology (equal); writing – original draft (equal).

Wendt Müller: Conceptualization (equal); methodology (equal); writing – review and editing (equal).

Bert Thys: Formal analysis (equal); writing – review and editing (equal).

Marcel Eens: Conceptualization (equal); methodology (equal); writing – review and editing (equal).

Acknowledgements

We thank Peter Scheys and Geert Eens for their field assistance. This work was supported by the University of Antwerp and FWO Flanders (FWO projects ID: G0A3615N and G052117N to ME and Rianne Pinxten). We thank both for their support.

Chapter 3

Intrinsic individual variation in daily activity onset and plastic responses on temporal but not spatial scales in female great tits

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Published in Scientific Reports, 2022, 12, 18022

https://doi.org/10.1038/s41598-022-22935-1

Abstract

In a variety of species, individuals appear to be consistent in the daily timing of their activity onset. Such consistent among-individual differences can result from both intrinsic factors, as individuals may e.g. differ genetically, and extrinsic factors, as the environment may vary on spatial and temporal scales. However, previous studies typically did not differentiate between their respective contributions on individual variation in the timing of activities. Here, we repeatedly measured the onset of activity in female great tits (*Parus major*) on consecutive days during the egg laying phase of the breeding season in four consecutive years. Subsequently, we used a variance partitioning analysis in order to determine which part of the total variation could be attributed to intrinsic (female identity) and extrinsic (nest box identity) factors. Overall, 27% of the total variation could be attributed to female identity. In addition, we found temporal variation in the activity onset, indicating that individuals can plastically adjust their timing. Yet despite their general ability to change the timing of activities over time, spatial environmental factors did not contribute significantly to the observed variation. Individuals may choose a habitat that matches the preferred timing of activities, or might not benefit from adjusting their timing to environmental factors that might vary on spatial scales.

Introduction

The timing of the daily active period differs tremendously among species, which has among others led to a categorisation of species being diurnal, nocturnal or crepuscular. From a functional or ecological perspective, the key drivers of this temporal niche partitioning in the timing of the active period are thought to be reduced competition among species and/or avoidance of predation (Carothers & Jaksić, 1984; Kronfeld-Schor & Dayan, 2003; Lesmeister et al., 2015). Also within species consistent differences among individuals in the timing of the onset of the active period have been reported. Although among individual variation is less pronounced than the variation among species (see Chmura et al. (2020) for an overview in birds), multiple studies have shown the existence of consistent individual differences in the timing of their active period, so called ‘chronotypes’ (Helm et al., 2017), in a variety of species. For example, free-ranging pearly razorfish (*Xyrithchys novacula*), Arctic ground squirrels (*Urocitellus parryii*) and blue tits (*Cyanistes caeruleus*) are repeatable in their activity onset and offset (Alós et al., 2017; Chmura et al., 2020; Schlicht & Kempenaers, 2020). Yet despite increasing evidence, not only the functional consequences, but also the underlying causes of this individual variation are largely unknown (Helm & Visser, 2010). Identifying the factors that underlie the above-mentioned consistent differences is therefore crucial for a deeper understanding of the ultimate causes of intraspecific variation in the timing of activity.

There are several possible underlying drivers that could lead to consistent differences in the timing of activities among individuals. On the one hand, intrinsic characteristics in the functioning of the biological clock may differ among individuals (Nikhil et al., 2016). For some species, it has been shown that genetic differences among individuals are related to differences in the functioning of the internal biological clock and that certain characteristics of the biological clock have a hereditary component (Allebrandt et al., 2010; Helm & Visser, 2010; Maukonen et al., 2020; Roecklein et al., 2012; Steinmeyer et al., 2012; Stuber et al., 2016). The biological clock, in turn, can influence the timing of activity and hence contribute to individual variation, although the exact mechanisms still need to be clarified (Helm et al., 2017). As a matter of principle, maternal effects, epigenetic effects or developmental plasticity may be responsible for intrinsic differences in the functioning of the biological clock as well, although little is known on this subject yet.

On the other hand, consistent differences in the environment in which individuals find themselves could equally lead to consistent differences in the timing of behaviour. These extrinsic factors may act on temporal as well as on spatial scales. On a temporal scale fluctuations in food availability have been shown to influence the timing of the dawn song in male birds (Cuthill & Macdonald, 1990; Grava et al., 2009; Saggese et al.,

2011) and higher temperatures are related to earlier activity onset in blackbirds (*Turdus merula;* Dominoni, 2013), but cause a slightly later onset of activity in great tits (*Parus major;* Lehmann et al., 2012). Because environmental factors can change over time it is likely that individuals are more consistent in the timing of activity in the short term than in the long term (Chmura et al., 2020; Zsebők et al., 2017), hence consistent individual differences may contain a temporal component.

On a spatial scale anthropogenic stressors, predation risk and (again) food availability could be key environmental drivers causing individual variation in activity patterns. Anthropogenic stressors, like artificial light at night and noise pollution can disrupt sleep and normal circadian rhythms and as a consequence affect activity patterns. Artificial light at night is related to earlier activity onset in great tits (Raap, Pinxten, et al., 2016) and blackbirds (Dominoni, 2013) and noise pollution leads to an earlier activity onset in great tits (M. L. Grunst et al., 2021). Fear of humans and perceived predation risk may affect activity patterns as well. In areas with high human disturbance mammals shift their active phase and become relatively more nocturnal (Gaynor et al., 2018), while higher predation risk is related to longer sleep duration in great tits (Stuber et al., 2014). As environmental factors vary on a spatial scale, it can be expected that individuals that are repeatedly measured in one location are more consistent in their behaviour than individuals that have moved (Niemelä & Dingemanse, 2017; Zsebők et al., 2017). Furthermore, food availability may not only change on a temporal scale, but can also differ spatially such as among territories, implying that spatiotemporal variation in environmental factors (partly) shapes activity patterns.

Spatiotemporal variation in environmental factors may thus cause consistent differences among individuals in the timing of activity onset that are not attributable to intrinsic characteristics. Although there are several indications that both intrinsic differences among individuals in the functioning of the biological clock and spatiotemporal variation in environmental factors can in interplay cause consistent differences in behaviour among individuals (Chmura et al., 2020; Niemelä &

Dingemanse, 2017; Zsebők et al., 2017), their relative importance in determining activity patterns is yet unknown.

Phenotypic variation is likely caused by processes operating at multiple levels, i.e. a certain phenotype is expressed by an individual from a given population at a given moment of time, and this necessitates to consider hierarchical structures (Garamszegi & Møller, 2017). Partitioning of variances can provide information on the different variance components and also allows to estimate their repeatability, which may improve our understanding of the evolutionary potential of a certain behavioural trait, here chronotypes. Yet, behavioural ecologists often try to relate an individual's mean trait value to fitness parameters like reproductive success, ignoring possible variation in labile traits within individuals, and how trait expression differs among hierarchical levels. Both behavioural traits and fitness parameters may co-vary with (changes in) the environment, which may result in the reporting of non-causal relationships and relationships that hold true on the within-individual level, but not on the among-individual level (Niemelä & Dingemanse, 2018). Revealing the relative contributions of intrinsic and extrinsic sources of variation, especially by distinguishing between spatial and temporal variation in the environment, may thus help to understand which factors shape behaviour and how to correctly measure it.

Here, we use longitudinal data to investigate the sources of among individual variation in activity onset in a nest box breeding population of great tits. To this end, we measured the timing of the first emergence from the nest box of female great tits during the egg laying phase of the breeding season in four consecutive years. As female great tits often breed in multiple years it is possible to determine repeatability of activity onset both in the short term (i.e. within years) and in the long term (i.e. between years). This provides insight into the importance of temporal variation in environmental effects in causing consistency in the timing of activity onset. Furthermore, environmental effects may add a spatial component, as some females breed multiple years at the same location (i.e. in the same nest box), while others switch from breeding location (i.e. they breed in a different nest box). Additionally, there are nest boxes which were used by multiple females over the years. This individual-based spatiotemporal information allows for the use of a variance partitioning analysis, enabling us to determine which part of the variation in activity onset in the population can be attributed to differences among individuals (i.e. intrinsic differences) and differences among nest boxes (i.e. extrinsic/environmental differences) in which individuals are measured (Browne et al., 2007; Niemelä & Dingemanse, 2017; Pettifor et al., 2003; Zsebők et al., 2017).

Materials and methods

All data were collected in a nest box breeding population of great tits, located in Wilrijk (Antwerp), Belgium (51°09’46.1"N, 4°24’13.3"E), during the breeding seasons of 2018, 2019, 2020 and 2021. In the study area about 170 nest boxes are available in trees at a height of about two meters. The population (covering ± 2.4 km^2) is located in a suburban area with a large amount of environmental variation in terms of light and noise pollution (Casasole et al., 2017), vegetation type and cover and ground surface hardening. Yearling female great tits are reproductively mature and have a life expectancy of 12 to 21 months, with a maximum of seven to 10 years (Payevsky, 2006; Vermeulen et al., 2017), so they often breed in multiple years. During the egg laying phase of the breeding season females lay one egg each morning for 5 to 13 days in a row in our population. In the morning females leave the nest box for the first time after laying the egg (Haftorn, 1981). In order to enable individual recognition all individuals were caught in the nest box during roosting in winter or during chick feeding in the breeding season and equipped with a PIT tag (passive integrated transponder; EM4102, 125 kHz, Eccel Technology Ltd, Aylesbury, UK) and a unique combination of colour rings. During the breeding season all nest boxes were checked regularly. When the nest building was completed nest boxes were checked every day, so the lay date of the first egg was known for all pairs. Emergence times (i.e. the first time a female leaves the nest box in the morning) were measured on multiple consecutive days during the egg laying phase. To minimise disturbance, nest boxes were not checked after the first egg was laid until all data were recorded. In our population females can have up to two broods per year, but in this study all emergence times were measured during the egg laying phase of the first breeding attempt only.

In order to obtain data on as many females as possible, emergence time from the nest box was measured using three different devices: SongMeters (SongMeterTM SM2 + ; Wildlife Acoustics, Inc, U.S.), radio-frequency identification (RFID) loggers (EM4102 data logger, Eccel Technology Ltd, Aylesbury, U.K.) and infrared sensitive cameras (Pakatak PAK-MIR5, Essex, UK, Grunst et al., 2022). SongMeters were placed on top of the nest box, with one microphone inside and the other microphone outside the nest box. Sound was recorded from 04:00 to 08:00 a.m. CET during the winter time period. After the clock changed to summer time sound was recorded from 03:00 to 08:00 a.m. CET. When females leave the nest box in the morning the sound of their wings can often be heard as well as their claws on the nest box (Halfwerk et al., 2011) and a change in air pressure when the female passes through the opening of the nest box. Avisoft SASLab Pro 5.2.14 was used to determine emergence time (Specht, 2002). RFID readers register PIT tagged individuals when they fly through the two antennas, which were placed around the nest box opening. Both the unique PIT tag number and the time of leaving/entering the nest box was saved (for more details see Iserbyt et al., 2018). Infrared sensitive cameras were installed under the lid of the nest box, pointing downwards. The cameras recorded immediately after installation at least 2 h before sunset and were switched off on collection the next morning at least 2 h after sunrise (Raap et al., 2015).

From the total of 1076 observations 5 datapoints were collected using the infrared cameras (1 in 2018, 4 in 2021), 49 using the RFID loggers (9 in 2018, 40 in 2020) and all remaining data via SongMeters (see also Meijdam, Müller, Thys, et al., 2022 for more details on the data selection process). We removed one datapoint from the dataset as it was an outlier. The emergence time was more than an hour earlier than all other datapoints. In total, data were collected on 162 females in 118 different nest boxes in 4 consecutive years. Within years emergence times were repeatedly measured on one to eight consecutive mornings per female (3.60 ± 1.22 times, mean ± s.d.), within one nest box. Between years females can switch nest boxes for breeding. Females were measured in one to four different years, in up to three different nest boxes (see Table 1 for a detailed overview of sample sizes for females). For nest boxes, emergence times were measured in one to four different years, with up to three different females (see Table 2 for a detailed overview of sample sizes in nest boxes). All emergence times were determined relative to sunrise on the day of measurement (negative = before sunrise, positive = after sunrise). Temperature data (measured every 30 min) were retrieved from a local weather station at the Antwerp international airport nearby our study population (± 5 km) via: https://www.wunderground.com/history/daily/be/antwerp. In our statistical analyses we used the temperature that was measured closest to sunrise.

Table 1: Overview of the number of different nest boxes and years in which females were measured. For example, there were 12 individuals that were measured in 3 different years in 2 different nest boxes (i.e. they moved once) and 9 individuals that were measured in 3 different years in only 1 nest box (i.e. they did not move). Repeated measures within years are not included in this table.

Number of different years in which a female was measured	Number of different nest boxes in which a female was measured			
	1	2	3	4
1	60			
2	40	32		
3	9	12	2	
4	2	2	3	0

Table 2: Overview of the number of years in which a nest box was occupied and the number of different females that were measured inside a nest box. For example, there were 2 nest boxes that were occupied in 4 different years with 3 different individuals, and 8 nest boxes that were occupied in 4 different years with only 2 different individuals. Repeated measures within years are not included in this table.

Number of different years in which emergence times were measured in a particular nest box	Number of different females that were measured in the nest box			
	1	2	3	4
1	8			
2	10	39		
3	6	39	4	
4	2	8	2	0

Statistical analyses

All statistical analyses were performed in R 4.1.1 (R Core Team, 2013). We used a linear mixed model with emergence time as the response variable and a polynomial date (mean-centred within years) effect up to the second order, the temperature at sunrise (mean-centred within years), year (as categorical variable) and age of the female (yearling breeder versus older birds [> 2 years]) as fixed effects. Female identity (FemaleID), nest box identity (NestID), the unique combination of FemaleID and the year (FemaleID_Year), and the unique combination of FemaleID and NestID (FemaleID_NestID) were included as random effects. FemaleID_Year was included in the model to be able to determine the variation in emergence times among years within individuals. FemaleID_NestID explains variation among individuals in how their emergence times change in response to different nest boxes and can thus be interpreted as differences among females in their plasticity with regard to emergence times (although part of this variation may also be explained by environmental changes in the surroundings of the nest boxes between years; see Discussion). We used the spaMM package (Rousset & Ferdy, 2014) to obtain 95% confidence intervals for all fixed and random effects by 1000 paramatric bootstrapping simulations.

We calculated the relative contribution of each random effect to the total variance (i.e. variance partitioning), including the 95% confidence intervals, based on this model. Following Araya-Ajoy et al. (2015), we calculated the short term (= within years) (Eqn 1) and long term (= between years) repeatability (Eqn 2) for emergence times.

$$R_{shortterm} = \frac{V_{FemaleID} + V_{FemaleID\_Year}}{V_{FemaleID} + V_{NestID} + V_{FemaleID\_NestID} + V_{FemaleID\_Year} + V_{Residual}} \qquad \text{(Eqn 1)}$$

$$R_{longterm} = \frac{V_{FemaleID}}{V_{FemaleID} + V_{NestID} + V_{FemaleID\_NestID} + V_{FemaleID\_Year} + V_{Residual}} \qquad \text{(Eqn 2)}$$

As FemaleID_NestID explains differences among individuals in plasticity, it contributes to the variation that is explained by intrinsic differences among individuals. Therefore, we also calculated the combined effects of FemaleID and FemaleID_NestID relative to the total variance (Eqn 3).

$$R_{intrinsic} = \frac{V_{FemaleID} + V_{FemaleID\_NestID}}{V_{FemaleID} + V_{NestID} + V_{FemaleID\_NestID} + V_{FemaleID\_Year} + V_{Residual}} \qquad \text{(Eqn 3)}$$

In order to compare the repeatability of emergence times between individuals that moved between nest boxes to breed in and individuals that did not move, we created two subsets. Linear mixed models were created for two subsets, which included the same fixed effects as described above, but this time only FemaleID and FemaleID_Year were included as random effects. Again we used the spaMM package to calculate the long term repeatability of moved ($n_{females}$ = 49) and not-moved individuals ($n_{females}$ = 69), and used 1000 parametric bootstrapping simulations to quantify 95% confidence intervals (Rousset & Ferdy, 2014).

Ethical note

This study was approved by the ethical committee of the University of Antwerp (ID numbers: 2016–87 and 2018–50) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complies with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licenses for all authors and technicians. Handling time was minimized as much as possible. All other methods described above are non-invasive.

Results

On average female great tits emerged from the nest box 8.9 min after sunrise (s.d. = 18.0 min, min = − 62 min, max = 91 min). Compared to 2018, females emerged 5.9 min earlier from the nest box in 2020 and 2.8 min later in 2021 (Table 3). Emergence times were affected by date (Figure 1, Table 3). Later during the breeding season emergence times became later relative to sunrise. Furthermore, older females (> 2 years) were slightly, but significantly, earlier than yearling females.

Table 3: Results from linear mixed effects model with emergence time (in minutes relative to sunrise) as response variable. Date and temperature (T) at sunrise were mean-centred within years. 95% confidence intervals are calculated with parametric bootstrapping.

Fixed effect	β	t	Lower 95% CI	Upper 95% CI
Poly(Centred Date)1	147.86	5.66	135.0	193.1
Poly(Centred Date)2	60.65	2.52	47.64	110.77
Centred T sunrise	-0.30	-2.06	-0.45	0.11
Year 2019	-2.06	-1.08	-3.96	0.38
Year 2020	-5.92	-2.82	-8.00	-3.51
Year 2021	2.75	1.10	0.08	5.107
Older/Yearling	-4.24	-2.66	-5.64	-2.30
Random effect	σ^2		Lower 95% CI	Upper 95% CI
FemaleID	79.46		58.42	104.64
NestID	19.42		1.74	36.45
FemaleID_Year	53.19		53.67	91.30
FemaleID_NestID	19.62		-0.95	53.11
Residual	119.40		107.8	131.5

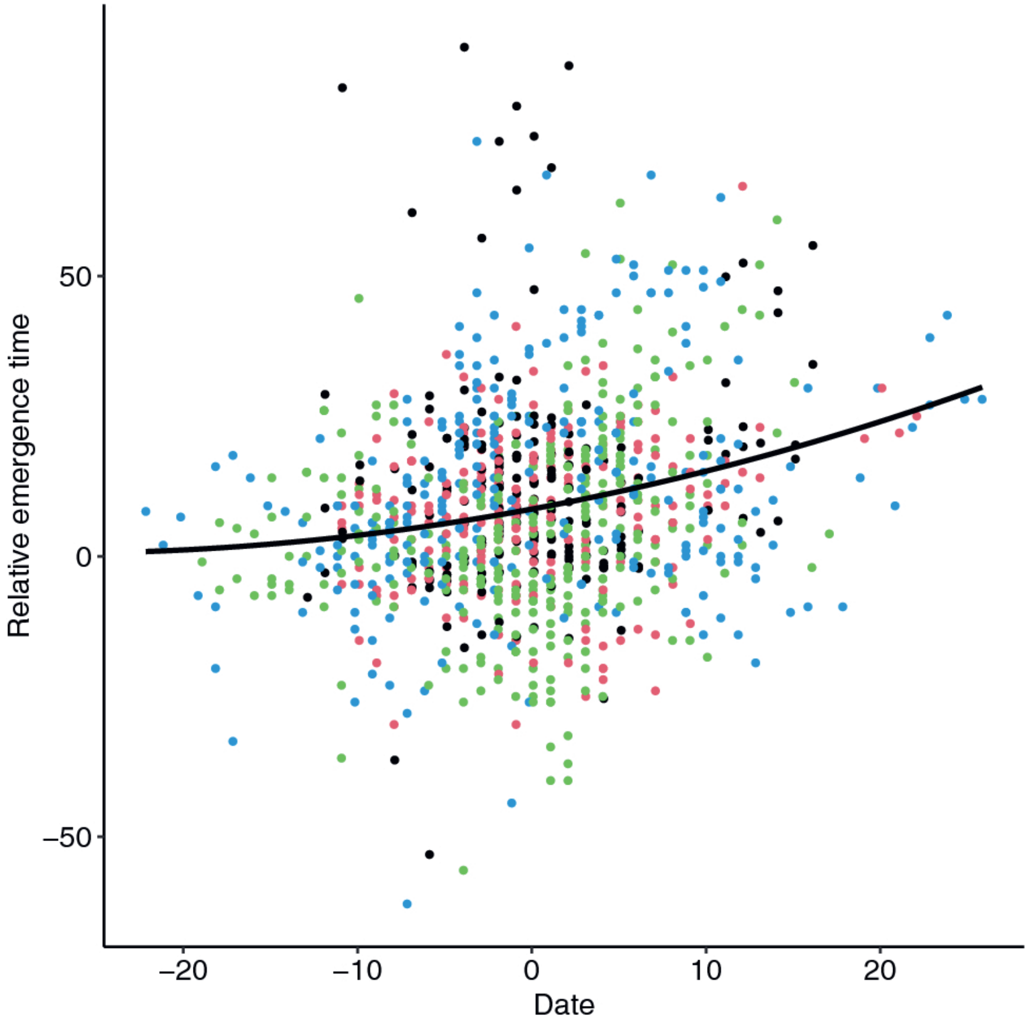

Figure 1: Date (mean-centred within years) positively affects relative emergence time (in minutes; negative = before sunrise, positive = after sunrise). Black = 2018, red = 2019, green = 2020, blue = 2021.

Both the short and long term repeatability for emergence time were significant, although the long term repeatability was lower than the short term repeatability ($R_{short\ term}$ [95% CI] = 0.46 [0.39, 0.59]; $R_{long\ term}$ = 0.27 [0.16, 0.34]). NestID and FemaleID_NestID did not explain significant parts of the total variation (Table 4). FemaleID and FemaleID_NestID together explained 34% of the total variation ($R_{intrinsic}$ = 0.34 [0.24, 0.43]). The long term repeatabilities of emergence time for females breeding in the same nest box and females that moved were very similar (R_{moved} [95% CI] = 0.29 [0.16, 0.38]; $R_{not\text{-}moved}$ = 0.29 [0.20, 0.36]; Figure 2; Tables S1, S2).

Table 4: The relative contribution of random effects to the total variance. 95% confidence intervals are calculated with parametric bootstrapping and are shown between brackets.

Random effect	Variance partitioning
FemaleID	0.27 [0.16, 0.34]
NestID	0.07 [-0.008, 0.12]
FemaleID_Year	0.18 [0.17, 0.31]
FemaleID_NestID	0.07 [-0.01, 0.19]
Residual	0.41 [0.32, 0.41]

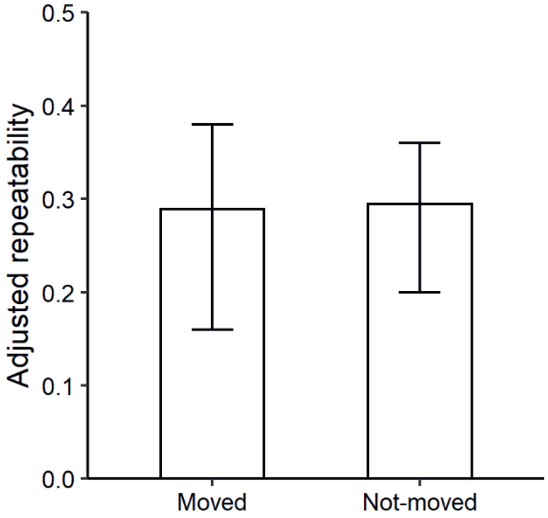

Figure 2: The long term repeatability of emergence time for females that did not move between nest boxes and females that moved (adjusted for year, age, date, date^2 and temperature at sunrise). 95% confidence intervals are indicated.

Discussion

In this longitudinal study we investigated among individual variation in activity patterns and how consistent differences among individuals in the timing of behaviour arise. To this end, we used a variance partitioning analysis. Emergence time as measured during the egg laying phase of the breeding season had a significant intrinsic component, being repeatable both within and between years. Among individual differences in plastic response to nest box changes (= FemaleID_NestID) did not contribute significantly to the total variation. The repeatability that was caused by intrinsic differences between individuals was thus mainly explained by among individual differences (= FemaleID). We also identified a temporal component in activity onset (= difference between short and long term repeatability), while environmental effects at the spatial scale (= NestID) appeared to be of less importance.

Temporal effects

We found a considerable difference between short and long term repeatability. This indicates that on short time scales the environment is more stable than on longer time scales, which causes individuals to behave more consistent in the short term than in the long term. This may be due to factors like weather conditions or prey abundance which often change slowly and are therefore more alike on short time scales. Furthermore, temporal autocorrelation of measurements on short time scales may also be caused by the internal state of an individual. For example, body condition typically varies little from day to day (Mitchell et al., 2020). Therefore, the variance within individuals will be relatively small in the short term and as a consequence the relative contribution of among-individual variance to the total variation (= repeatability) will be larger (Bell et al., 2009; Zsebők et al., 2017).

As most studies do not take temporal variation in the environment into account when studying repeatability of activity onset, many studies on repeatability of activity onset have reported estimates that are similar to or exceed our short term repeatability estimate (Dominoni, 2013; Graham et al., 2017; M. L. Grunst et al., 2021; Maury et al., 2020; Schlicht et al., 2014; Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010). However, Schlicht et al. (2014) tried to differentiate between short and long term repeatability of activity onset in blue tits (here mean emergence time within years for each female was used to calculate long term repeatability). Between years a non-significant repeatability estimate of 0.15 was found (Schlicht et al., 2014). Furthermore, Stuber, Dingemanse, et al. (2015) reported a repeatability estimate of 0.13 for activity onset in great tits. Here, no statistical distinction was made between short and long term repeatability, but the interval between measurements was much larger than in most other studies (months or a year instead of days). In Arctic ground squirrels repeatability estimates of activity onset decreased when the number of consecutive sampling days increased (Chmura et al., 2020). Altogether, this suggests that temporal autocorrelation of datapoints can influence repeatability estimates.

In captivity, short term repeatability of activity onset for male great tits was 0.40 (Lehmann et al., 2012), which is fairly similar to our short term estimate. However, in captivity temporal variation in the environment is much smaller, so the repeatability is not expected to decrease as much over time. The lower long term repeatability estimate we found in free-living great tits seems to further underline that individuals may plastically adjust the timing of activity onset to environmental factors that vary on a temporal scale (although the sexes may differ in repeatability as well).

Spatial effects

Spatial variation in the environment did not explain a significant proportion of the total variation in emergence time. In another great tit population and in blue tits there were also no consistent differences among nest boxes in activity onset (Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015). This result was nevertheless unexpected, as for example artificial light at night and noise pollution levels, which are known to affect activity onset (Dominoni, 2013; M. L. Grunst et al., 2021; Raap, Pinxten, et al., 2016), vary throughout our suburban study population (Casasole et al., 2017). One possible explanation for the absence of a spatial component may be that when great tits move between nest boxes, they very often stay in the vicinity of their previous nest box, so that the environmental differences between the previous nest box and the new one may be small (only one individual moved more than two nest boxes further). It is therefore possible that environmental effects act on a larger spatial scale, and remain hence undetected in our study. However, we think that this might rather be unlikely, given the substantial differences already being present at a local scale. A visual inspection of emergence times on a map of our nest boxes indeed did not reveal obvious spatial patterns in emergence times, e.g. in the context of urbanisation. Nest boxes may also (partly) shield individuals from environmental factors like artificial light and noise pollution (Raap et al., 2018), particularly in females that sleep in their nest box during the entire breeding season. Furthermore, phenotype habitat matching (Edelaar et al., 2008), where individuals settle in certain habitats in a non-random way, could explain the minor contribution of spatial variation to the total variation. For example, individuals that are more easily disturbed by anthropogenic stressors, such as artificial light at night and noise pollution, may only choose nest boxes with low levels of these stressors. Such non-random settlement would cause a limited amount of environmental variation between the different nest boxes in which an individual chooses to breed. Another possibility may be that certain factors force individuals to become active within a specific time window, for example to avoid competition for food or to minimise predation risk, which makes it impossible to adjust their behaviour to the local conditions. Finally, it is possible that spatial variation in the environment among nest boxes is not constant over time, but that it fluctuates among years, so that environmental effects on a spatial scale (NestID effect) do not contribute substantially to the variation. Instead, this spatiotemporal variation may show up in the differences among individuals in their plastic response to different nest boxes (FemaleID_NestID effect; see below).

Individual differences in plastic response

Differences among individuals in their temporal plasticity (= change in activity onset in function of nest box changes) did not explain a significant part of the variation observed. This is intriguing, because previous research on great tits showed a large variation in how individuals changed their emergence time after exposure to artificial light at night inside a nest box (Raap, Pinxten, et al., 2016). As nest boxes differ in light exposure, one could have expected to find differences among individuals in their plasticity. However, as mentioned above, emergence times were only measured for females that slept inside a nest box and nest boxes can shield individuals from light exposure (Raap et al., 2018).

In order to determine which part of the total variation in activity onset was caused by intrinsic differences among individuals we determined the combined effects of FemaleID and FemaleID_NestID relative to the total variance. However, in our dataset, nest box changes occurred across years, so that there is a significant temporal component as well. That is, part of the variation explained by differences among individuals in their plastic response to different nest boxes may actually be due to spatial fluctuations between years in the environment. Our great tit population is suburban and therefore very dynamic (e.g. due to changing construction sites) and spatial patterns of anthropogenic stressors may have changed between years. Furthermore, females can change not only their nest box, but also their partner between years (83% of the movements between nest boxes, where the males were known in both nest boxes, were associated with a change of partner). Before females leave the nest box in the morning they often communicate vocally with their partner (Gorissen & Eens, 2004; Halfwerk et al., 2012; audible on SongMeters). Therefore, the timing or the quality of the partner's dawn song may affect female emergence times. However, Steinmeyer et al. (2013) found a correlation between awakening times of blue tit partners that were standardised within days per sex, so that relatively early males mated with relatively early females, but no significant correlation was found between the absolute awakening times (i.e. not standardised), which may suggest there is no direct effect of the male's awakening time on the female's emergence time (Steinmeyer et al., 2013). Including male identity in our model might have revealed whether there may be other mechanisms by which the male affects female emergence time (e.g. male quality or song characteristics), but unfortunately, we did not have enough data to estimate the male's contribution properly.

Finally, despite our large dataset on female emergence times, it has to be taken into account that the number of females that moved was relatively low. Therefore, it is possible that our dataset did not contain enough information to properly estimate differences among individuals in their temporal plasticity.

Within individual variation

In this study emergence times were determined only during the egg laying phase of the breeding season and during this period females may be more consistent in the timing of their activity onset than during other periods. As the daily timing of egg laying seems to be under control of the biological clock (J. R. Cain & Wilson, 1974; Zhang et al., 2016) and females always leave the nest box after laying the egg (Haftorn, 1981), there may be constraints to lay and thus to emerge earlier. This may result in smaller variation within individuals during the egg laying phase than during other periods and consistency of activity onset may thus be larger during the egg laying phase.

Chronotypes – consistent daily activity patterns in a changing world

We performed this study in a great tit population that is located in a suburban area with a large amount of environmental variation, e.g. in terms of light and noise pollution. We therefore expected to find an effect of the nest box reflecting this spatial variation (see also Dominoni, 2013; Womack, 2020). However, we did not find any indications that spatial variation affected emergence times in female great tits sleeping in nest boxes. This suggests that great tits might be resilient to light and noise pollution—at least as long as they sleep in nest boxes or similarly sheltered nests, since experimental studies showed that exposure to both light and noise pollution affect the start of activity in great tits (Dominoni et al., 2020; M. L. Grunst et al., 2021; Raap et al., 2015). Whether urbanisation poses a challenge for timing in great tits may thus depend on their choices of nest locations and the availability of cavities to roost in.

Not only urbanisation, but also climate change may affect the timing of activity in great tits. In recent years, egg laying dates in great tits have significantly advanced (e.g. in Belgium Matthysen et al., 2011) and earlier laying dates are associated with late sunrise and late onsets of activity. Individuals may thus have to adjust the timing of activity onset so that they are not restricted by the shorter daylengths, e.g. in the time they need to forage. Here, we found relatively large amounts of residual variation and variation within individuals between years, which indicates that there might be sufficient plasticity in emergence times. However, at some stage there may be limitations to becoming active earlier, for example because of problems with vision in the dark, so for how long great tits can keep track of advancing spring conditions for egg laying remains to be seen.

Conclusion

Repeatability of activity onset has been determined many times in several bird species. However, to the best of our knowledge, never before have temporal and spatial variation in the environment been taken into account at the same time in these analyses. Neither have among-individual differences in the plasticity of timing been taken into account. Here, we show that emergence time in free-living female great tits has a significant intrinsic component and may hence be subjected to both sexual and natural selection pressures (Helm et al., 2017). Surprisingly, female great tits did not adjust their behaviour to environmental variation on spatial scales. At the same time we show that the timing of activity onset varies over temporal scales and that the variation within individuals is relatively large. Activity onset in female great tits is thus a rather plastic trait, which may be sensitive to the prevailing environmental conditions, although the exact components still need to be identified.

Supplementary material

The supplementary information accompanying this chapter can be found on pages 178 – 179 of this thesis and is available online at DOI:10.1038/s41598-022-22935-1

Author contributions

W. M., M. E. and M. M. conceived the study. M. M. performed the field work, collected the data and wrote the manuscript under supervision of W. M. and M. E. Statistical analyses were performed by M. M. All authors contributed in revising the manuscript and gave final approval for publication.

Acknowledgements

We thank Peter Scheys and Geert Eens for their field assistance and Bert Thys and Alexandre Courtiol for their valuable advice on the data analyses.

Chapter 4

Artificial light at night impairs inhibitory control in a wild songbird

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Published in Science of The Total Environment, 2023, 885, 163765.

https://doi.org/10.1016/j.scitotenv.2023.163765

Abstract

Anthropogenic stressors, such as artificial light at night (ALAN), increasingly affect the sleep behaviour and physiology of wild birds, particularly in areas where human activity is prevalent. To understand the consequences of the resulting sleep deprivation, it is essential to investigate whether the effects of sleep deprivation on cognitive performance, observed in humans, also occur in birds. Here, we studied the impact of sleep deprivation, induced by intermittent exposure to ALAN, on inhibitory control, vigilance behaviour, and exploratory behaviour in great tits. Furthermore, we hypothesised that the effect of ALAN could depend on an individual's natural sleep duration and the timing of sleep. To achieve these goals, we measured emergence and entry times from/into the nest box in the wild, before capturing the great tits. In captivity, half of the birds were exposed to intermittent ALAN, and cognitive performance was assessed the following morning for all birds. ALAN-exposed birds were less successful on the detour reach task and when they started pecking at the test tube, they pecked more often. However, neither of the effects was related to the natural sleep duration or timing, in contrast to our hypothesis, and there were no differences between the ALAN-exposed and non-exposed group in vigilance and exploratory behaviour. Thus, even one night of exposure to ALAN can negatively affect cognitive performance in wild birds, possibly with negative effects on their performance and survival.

Introduction

Sleep is found in a wide variety of species (Siegel, 2008) and, despite being ubiquitous in the animal kingdom, its various possible functions have been debated for years (Anafi et al., 2019; Assefa et al., 2015; Krueger et al., 2016). To shed light on these functions, sleep deprivation experiments have been conducted in humans, revealing numerous negative effects, including reduced energy levels (Jung et al., 2011), increased negative and decreased positive moods (Tomaso et al., 2021), immunosuppression (Garbarino et al., 2021), and it is particularly known to impair cognitive performance (Orzeł-Gryglewska, 2010). Even one night of sleep restriction can strongly reduce cognitive functions such as memory, alertness, problem solving and inhibitory control (e.g. Frenda & Fenn, 2016; Grant et al., 2017; Ho et al., 2015; Horger et al., 2021). However, most studies investigating the impact of sleep deprivation on cognitive performance are limited to humans and a handful of other mammalian species, leaving significant uncertainty regarding the generalisability of these findings across the animal kingdom. Given that the structure of avian sleep is similar to that of mammals, its functions may be comparable and birds may thus experience similar effects of sleep deprivation on cognitive performance as mammals (Lesku et al., 2011; Rattenborg et al., 2009). Surprisingly, in some bird species sleep deprivation seems to have no apparent effects at all during periods in which ecological demands favour wakefulness, for example during migration or in the breeding season (Lesku et al., 2012; Rattenborg et al., 2004; see also Lesku & Rattenborg, 2022). Better knowledge of the consequences of sleep loss in non-mammalian species is hence urgently needed, especially in the light of the (potential) increasing impact of anthropogenic stressors on sleep in wild animals.

Human activity covers an increasingly significant portion of the world, which is often accompanied by anthropogenic stressors such as artificial light at night (ALAN) and noise pollution (Goines & Hagler, 2007; Hölker et al., 2010). Recent studies in birds revealed that these anthropogenic stressors have adverse effects on sleep, leading among others to shorter sleep duration and earlier wake up times (Aulsebrook, Connelly, et al., 2020; Aulsebrook, Lesku, et al., 2020; Connelly et al., 2020; M. L. Grunst et al., 2021; Raap et al., 2015; Raap, Pinxten, et al., 2016; Yorzinski et al., 2015). This may result in severe sleep deprivation. Even after one night of light exposure, birds show a sleep rebound (Aulsebrook, Connelly, et al., 2020; Raap, Pinxten, et al., 2016). This suggests that partial sleep deprivation has an impact that needs to be compensated for, but the possible consequences for cognitive performance are still unclear.

In birds, there are some indications for a link between sleep and cognitive functions, including imprinting, song learning, auditory discrimination and spatial learning (reviewed by Aulsebrook et al., 2021; Taufique, 2022). However, only a handful of studies have investigated the direct effects of sleep deprivation on cognitive performance, and although the number of studies is still very limited, the results are not necessarily consistent. For instance, sleep deprived Australian magpies (*Cracticus tibicen*) performed worse on a reversal learning task than non-deprived individuals (Johnsson et al., 2022) and white crowned sparrows (*Zonotrichia leucophrys gambelii*) performed worse on a repeated acquisition task after a night of sleep restriction during the non-migratory season, but not during the migratory season (Rattenborg et al., 2004). However, sleeping under green or white light did not affect learning and performance in memory tasks in great tits (Ulgezen et al., 2019). Thus, there is an urgent need for studies investigating the effects of sleep deprivation on cognitive functions in birds.

When studying impairment of cognitive performance by sleep deprivation in wild bird species, it is furthermore important to select traits that are biologically or ecologically relevant and that can be measured in a reliable way. Inhibitory control, which enables individuals to adjust an action that has already been initiated, is likely ecologically relevant as for example foraging behaviour should be inhibited when predators are present. In non-human animals inhibitory control is often measured using a detour reach task, during which an individual should take a reward from a transparent tube (reviewed by Kabadayi et al., 2018; MacLean et al., 2014). When an individual's inhibitory control is low, the individual tends to approach the reward directly, but it will face the closed side of the tube, so it starts to peck at the tube. Individuals with higher inhibitory control will be able to inhibit the initial response to approach the reward directly and will instead make a detour to the open end of the tube, which enables them to collect the reward. Vigilance is another behaviour that is extremely relevant for survival in birds, as it is the ability to sustain attention to potential threats or danger. Vigilance can be directed towards predators and conspecifics and can be quantified by the percentage of time spent on scanning the environment during a predetermined time frame (Beauchamp, 2015) or activity, such as foraging (e.g. Baker et al., 2011; Klett-Mingo et al., 2016). Since sleep deprivation impairs inhibitory control and vigilance in humans (Chuah et al., 2006; Magnuson et al., 2022; Mao et al., 2021; reviewed in Hudson et al., 2020 and Lim & Dinges, 2008) it is possible that anthropogenic stressors, such as ALAN and noise pollution affect both traits in birds due to sleep deprivation.

Although cognitive performance is affected by sleep deprivation, at least in humans, the extent of this effect may not be consistent across all individuals. There is for example significant among individual variation in the reduction of inhibitory control after sleep deprivation (Chuah et al., 2006). Interestingly, there are some indications that this may be related to an individual's habitual sleep rhythm, where individuals that sleep longer suffer more from sleep deprivation (Demos et al., 2016) and individuals with early chronotypes (i.e. individuals that go to sleep and rise early) may be affected differently than individuals with late chronotypes (i.e. individuals that go to sleep and rise late; Barclay & Myachykov, 2017; J. Song et al., 2019). For example, reaction times on a test for visual attention were longer after sleep deprivation in individuals with late chronotypes, but shorter in individuals with early chronotypes (Barclay & Myachykov, 2017). Differences between individuals in their natural sleep-wake cycles may thus determine to which extent cognitive performance is affected by sleep deprivation.

Given the increasing exposure to night-time anthropogenic stressors in the wild (Hölker et al., 2010), we aim to investigate whether cognitive performance is affected by exposure to ALAN in wild great tits. We focus on inhibitory control as measured in a detour reach task and vigilance during foraging. We predict that ALAN-exposed individuals are more likely to fail at successfully detouring without pecking during a detour reach task than non-exposed individuals and that they will be less vigilant, which may ultimately increase predation risk. Prior to the experiment, we measured sleep timing in the wild to investigate whether the effect of sleep deprivation depended on the natural sleep rhythm, in order to identify sources of variation in the effects of sleep deprivation on cognitive performance. In addition, we investigated whether there were differences between ALAN-exposed and non-exposed individuals in exploratory behaviour, which may affect cognitive performance (e.g. Gomes et al., 2020; Lucon-Xiccato et al., 2020), and can be altered by sleep deprivation or fragmentation (Tartar et al., 2009). Investigating the consequences of ALAN on cognitive performance in great tits may clarify the impact of anthropogenic stressors on wild birds.

Materials and methods

Study population

This study was carried out in two nest box populations of great tits (referred to as Fort7 and CDE), located in Wilrijk (Antwerp), Belgium. The populations were located at approximately 2 km distance from another. CDE is the southern campus of the University of Antwerp, which is publicly accessible and located in a suburban area, while Fort7 is a historical site that is embedded in the urban matrix but not accessible for the public and thus less disturbed. The experiment was carried out in January and February 2022. In both populations individuals were equipped with a ring containing a PIT-tag (passive integrated transponder; EM4102, 125 kHz, Eccel Technology Ltd., Aylesbury, UK) during roosting in the nest boxes at night. These PIT-tags could be detected by a handheld transponder reader (GR-250 RFID Reader, Trovan, Aalten, Netherlands), so birds could be located while roosting inside the nest boxes without being disturbed.

Sleep behaviour in the wild

We determined the timing and duration of sleep and activity of all individuals inside the nest box by measuring the emergence time from the nest box in the morning and the entry time in the evening on four or five consecutive days using SongMeters (SongMeter™ SM2+; Wildlife Acoustics, Inc., U.S.). Each SongMeter has two microphones to record sounds both inside and outside the nest box. Both microphones produce sonograms. Morning emergence time from the nest box and evening entry time could be determined by the sound of the bird's claws on the nest box (microphone inside) and the sound of its wings when taking off (microphone inside and outside). Furthermore, a specific sound caused by a change in air pressure could be heard when the bird passed through the opening of the nest box (Halfwerk et al., 2011; Meijdam, Müller, & Eens, 2022; Meijdam, Müller, Thys, et al., 2022). The SongMeters were programmed to record between 06:30 and 09:30 AM and between 04:00 and 06:30 PM. Emergence and entry times were measured for 54 individuals which were spilt into three groups. The first group was measured from January 21 to January 26, the second group from February 2 to February 7 and the third group from February 10 to February 14 (Table 1). Two microphones broke, so we had no data on two individuals from CDE (n_{Fort7} = 12, n_{CDE} = 40).

Lab conditions and sleep deprivation

Per test day, six individuals (in total 54) were captured in their nest boxes after sunset and were brought to the lab around 06:00 PM, where they spent one night and morning before being released. In the lab, birds were individually housed (0.83 × 0.4 × 0.5 m), they had ad libitum access to water and received 4 mealworms and 6 sunflower seeds. Birds were in auditory but not visual contact. In order to minimize stress, half of the front side of each cage was covered with a sheet of paper. At 07:00 PM the lights were turned off. Each group of 6 birds was assigned to either the ALAN-exposed or the non-exposed group (Table 1). For the non-exposed group the lights remained turned off until 07:30 AM. For the ALAN-exposed group the light was turned on for 15 min per hour from 09:00 PM until 04:15 AM (i.e. 8 times 15 min = 2 h of light). The light the birds were exposed to had a colour temperature of 3000 K (warm-white), and the amount of lux measured on the floor of the cages was 489 lx. However, within cages the amount of lux varied considerably depending on the exact position. Given that the sheet of paper that covered half of the cage was already present during the night the birds had the option to move to a darker spot. In the darkest spot the amount of lux on the floor was 138 lx. From 04:15 AM the lights remained turned off until 07:30 AM. For both groups the constant background noise was 31.7 dB. In order to check whether the light treatment resulted in sleep deprivation, an infrared sensitive camera (Pakatak PAK-MIR5) was installed in one of the cages, so per night the sleep behaviour of one individual could be recorded. Sleep behaviour was filmed for four individuals that were exposed to ALAN and for three individuals that were not exposed to ALAN (see Discussion section). All tests were performed in the same order (i.e. 1. Exploration test, 2. Detour reach task, 3. Vigilance test) the morning after capture. There were no breaks between tests and the birds remained food deprived except for the food that was eaten during the tests. After all tests were finished the birds' sex and age (yearling vs older) were determined based on plumage characteristics. Individuals were released back in the wild between 12:30 and 13:30 in the vicinity of the nest box where they were captured.

Table 1: overview of the sampling design.

Test Date	Population	Number of birds	Recording of sleep behaviour in the wild (date)	Experimental group
01/02/2022	Fort7	6	21/01/2022 – 26/01/2022	ALAN-exposed
02/02/2022	Fort7	6	21/01/2022 – 26/01/2022	Non-exposed
08/02/2022	CDE	6	02/02/2022 – 07/02/2022	ALAN-exposed
09/02/2022	CDE	6	02/02/2022 – 07/02/2022	Non-exposed
10/02/2022	CDE	6	02/02/2022 – 07/02/2022	ALAN-exposed
11/02/2022	CDE	6	02/02/2022 – 07/02/2022	Non-exposed
15/02/2022	CDE	6	10/02/2022 – 14/02/2022	ALAN-exposed
16/02/2022	CDE	6	10/02/2022 – 14/02/2022	Non-exposed
17/02/2022	CDE	6	10/02/2022 – 14/02/2022	Non-exposed

Exploratory behaviour

Before the exploration tests started all food was removed from the individual home cages. The tests started between 08:15 and 08:45 AM. The individual home cages were connected to an exploration room (4.0 × 2.4 × 2.3 m) containing five artificial trees (Dingemanse et al., 2002; Thys et al., 2017). Individual birds ($n_{\text{ALAN-exposed}} = 24$, $n_{\text{non-exposed}} = 30$) were released into the exploration room via a sliding door, by turning on the lights in the exploration room, while turning off the lights in the home cage. The sliding door to the home cage was closed from outside. The observer scored the behaviour from the observer room next to the exploration room, behind a one-way window. Exploration scores were calculated as the total number of hops (= movements within trees) and flights (= movements between trees, the floor and walls) within the first 2 min (for more details see Dingemanse et al., 2002). After 2 min the lights in the exploration room were turned off, the sliding door was opened, the light in the home cage was turned on and the experimenter entered the exploration room, so that the bird flew back into its own home cage.

Detour reach task

The detour reach task was performed in the home cages using transparent plastic tubes (13 cm in length, 4 cm in diameter), which were secured to a wooden block. The tubes were already present in the individual home cages upon the arrival of the birds into the test room at night, enabling birds to habituate to this novel object before the tests started. During the detour reach task a divider was used to lock the bird in one half of the cage behind the sheet of paper, allowing the tube to be manipulated without the bird seeing the experimenter (Figure 1). To further habituate the birds to the tube a euthanised mealworm (by compressing the head) was placed in front of the tube. This was repeated twice with a 15-min interval. Only birds that ate at least one of two mealworms continued to the test phase ($n_{\text{ALAN-exposed}} = 16$, $n_{\text{non-exposed}} = 19$). Unlike previous studies, we did not include a training phase in which individuals had to retrieve a worm from an opaque tube in order to learn the correct detour solution (e.g. Boogert et al., 2011; Davidson et al., 2022; Troisi et al., 2021), since we used wild individuals that had to be released in the early afternoon after capture. During the test phase a mealworm was placed inside the tube and birds were given 5 min to collect the mealworm. This test was repeated 8 times. During the tests individual birds were filmed (Sony DCR-SX 30) and their behaviour was analysed later. We determined for each test whether the mealworm was collected or not and how many times an individual pecked at the tube. To count the number of pecks, the movies were played at 0.3 times the normal speed. A trial was successful if the bird collected the worm without pecking at the tube, while it was considered unsuccessful if the bird collected the worm after pecking at the tube. Sometimes birds also pecked at the tube without managing to collect the worm, which was considered an invalid trial.

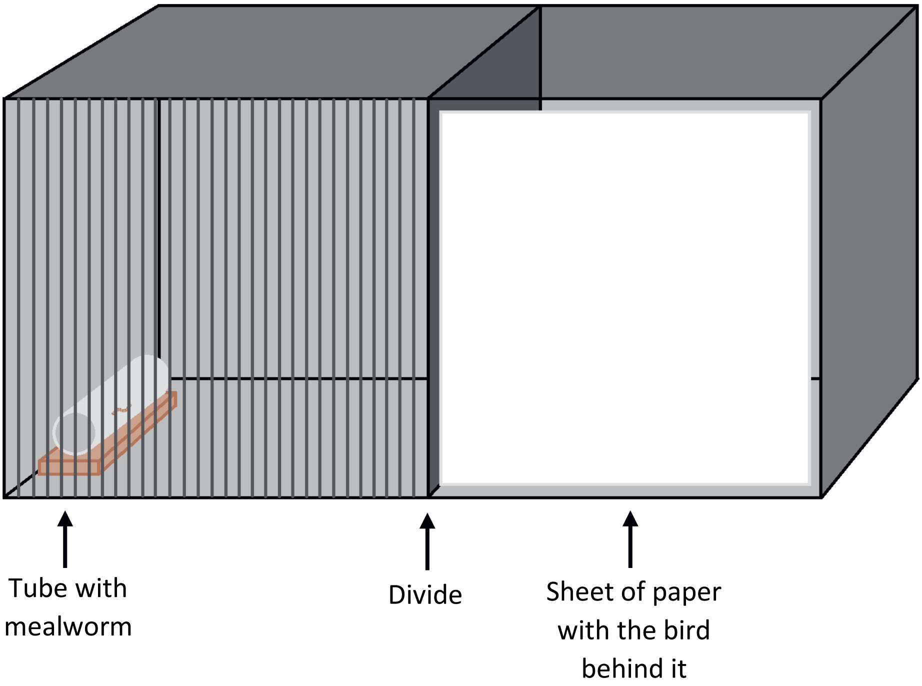

Figure 1: Set up in the individual home cage during the detour reach task.

Vigilance behaviour

A wire mesh feeder was placed against one wall of the exploration room. To increase manipulation time and ensure that birds remained on the feeder, whole peanuts were placed in the feeder that required the birds to peck at them. Individual birds (*n* = 54) were released into the exploration room (as described above) and their behaviour on the feeder was filmed for 10 min. Using these movies we analysed their vigilance behaviour. Two individuals were excluded from further analyses as they did not feed on the peanuts. For the remaining individuals we determined the first period of 20 s during which the individual was foraging on the peanuts without interruption (n_{ALAN-}

$_{\text{exposed}}$ = 24, $n_{\text{non-exposed}}$ = 28), after the experimenter had left the observer room next to the exploration room. During these 20 s we measured the percentage of time the individual spent on scanning the environment, by playing the movies at 0.1 times the normal speed. We defined scanning as the act of turning the head to inspect the environment and as birds were filmed from the back we used the visibility of the eyes as a reference point for scanning behaviour. We also counted the number of scans and foraging bouts performed by each bird during the 20 s period (see Klett-Mingo et al., 2016). All behaviours were analysed using the software JWatcher (Worthington & Egge, 2009).

Statistical analyses

All statistical analyses were conducted using R 4.1.3 (R Core Team, 2013). We used the lmerTest package to obtain *P*-values for fixed effects (Kuznetsova et al., 2017). Due to broken microphones, data on sleep behaviour in the wild was missing for two individuals, and they were removed from the dataset. The final sample sizes were $n_{\text{ALAN-exposed}}$ = 24 and $n_{\text{non-exposed}}$ = 28 for exploratory behaviour, $n_{\text{ALAN-exposed}}$ = 16, $n_{\text{non-exposed}}$ = 19 for inhibitory control and $n_{\text{ALAN-exposed}}$ = 24, $n_{\text{non-exposed}}$ = 26 for vigilance behaviour. The data from the exploration test were strongly right skewed and almost 20 % of the individuals scored zero. To test whether the exploration score differed between the ALAN-exposed and non-exposed group we used a negative binomial generalised linear model. The model included the group as fixed factor and we used a forward stepwise selection procedure based on the Akaike Information Criterium (AIC) value, to investigate whether the following fixed effects significantly improved the model: sex, age (yearling/older), test date, population (Fort7/CDE), the start time of the test, the sleep duration, emergence time and entry time under natural circumstances (mean centred within days and averaged per individual) and the interactions between experimental group and population, experimental group and sleep duration, experimental group and emergence time and group and entry time. The final model contained experimental group and population as fixed factors (see Table S1 for results on fixed effects that were not included in the final model; Table S2).

To test whether the number of scans during the vigilance test differed between the experimental groups we first tried a generalised linear model with a Poisson distribution, but as there was overdispersion we switched to a negative binomial generalised linear model. Again we used AIC values to compare models and the same fixed effects as described above for the exploration test were included. The final model contained experimental group and start time of the vigilance test (see Table S3 for results on fixed effects that were not included in the final model; Table S4). In addition to the number of scans we also tested whether the percentage of time spent scanning was affected by ALAN-exposure. Here, we used a linear model, which also contained experimental group and the start time of the vigilance test as fixed effects in the final model (see Table S5 for results on fixed effects that were not included in the final model; Table S6).

To test for differences in the scores on the detour reach task between the ALAN-exposed and the non-exposed group we calculated the proportion of successful trials per individual and used a generalised linear model with a binomial distribution. A successful trial was defined as a trial in which the bird collected the worm without touching the tube. In failed trials the bird collected the worm after pecking at the tube. Trials in which the worm was not collected were invalid. The weights were specified as the total number of tests during which the individual managed to collect the worm. Again models with the same fixed effects as described above, except for start time of the test, were compared based on AIC values, but this time we also included the amount of worms eaten during the habituation phase of the detour reach task (1 or 2). The final model included experimental group, population and entry time into the nest box under natural circumstances as fixed effects (see Table S7 for results on fixed effects that were not included in the final model; Table S8). Additionally, we tested whether individuals improved over trials and whether there were differences between the ALAN-exposed and non-exposed group in improvement over trials. Therefore, we repeated the analysis with a generalised linear mixed model that also included the trial (1–8, as a continuous variable) and the interaction between trial and group as fixed effects and the individual identity as random effect (see Table S9 for results; Table S10; Table S11). For the models on the proportion of successful trials during the detour reach task and the score (success/fail) on the detour reach task adding 'population' and 'date' gave comparable results (i.e. the difference was <2 AIC). Yet, continuing with population instead of date gave more parsimonious results, so we decided to not include date in the model.

Furthermore we tested whether there was a difference between the ALAN-exposed and the non-exposed group in the amount of pecks at the tube. We removed one datapoint as the amount of pecks was extremely high (487, while the second highest amount of pecks was 155). All trials in which the worm was taken and trials in which the worm was not taken, while the bird did peck at the tube were included. As negative binomial mixed models with individual identity as random effect could not converge, we calculated the average amount of pecks per individual. These data were not normally distributed, continuous, and contained many zeros. Therefore, we used a Wilcoxon-test to compare the ALAN-exposed and the non-exposed group. Since successful trials were included in this test, the result may reflect difference between the groups in the detour reach task score. Therefore we also examined the pecking behaviour for all trials in which individuals engage in pecking at all, and excluded all instances of non-pecking. We calculated the average amount of pecks per individual. Again the data was strongly right skewed, but as no zeros were included in this dataset we log-transformed the data to obtain a normal distribution. We compared linear models that included the same fixed effects as the model for the detour reach task score, except for trial and the interaction between group and trial, based on AIC values and selected a model that contained experimental group and entry time into the nest box under natural circumstances as the most parsimonious model (see Table S12 for results on fixed effects that were not included in the final model; Table S13).

Ethical statement

This study was approved by the ethical committee of the University of Antwerp (ID number: 2018-50) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complies with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licenses for all authors and technicians. Handling time was minimised as much as possible.

Results

Exploratory behaviour

On average individuals had an exploration score of 10.8 (±s.d. = 9.0, min = 0, max = 32). There were no differences in exploration scores between the ALAN-exposed and the non-exposed group ($\beta \pm SE = 0.03 \pm 0.29$, $z = 0.12$, $P = 0.91$, $n_{\text{ALAN-exposed}} = 24$, $n_{\text{non-exposed}} = 28$). Individuals from the Fort7 population were less exploratory than individuals from CDE ($\beta \pm SE = -0.96 \pm 0.36$, $z = -2.69$, $P < 0.01$, $n_{\text{Fort7}} = 12$, $n_{\text{CDE}} = 40$).

Detour reach task

On average individuals managed to collect the worm in 5.8 out of 8 trials (±s.d. = 1.7, min = 1, max = 8) of which 62 % was collected successfully (i.e. without pecking at the tube; mean ± s.d. = 0.62 ± 0.33, min = 0, max = 1). Individuals in the ALAN-exposed group and individuals from the Fort7 population were less successful in collecting the worm without pecking at the tube (Table 2, Figure 2). Individuals with later entry times were more likely to successfully collect the worm.

Table 2: Results from a generalised linear model with a binomial distribution, with the proportion of successful trials in the detour reach task as dependent variable ($n_{ALAN\text{-}exposed} = 16$, $n_{non\text{-}exposed} = 19$; $n_{CDE} = 27$, $n_{Fort7} = 8$).

Fixed factor	β	SE	t-value	P-value
Group Non-exposed[a]	0.89	0.33	2.66	0.008
Population Fort7 [b]	-1.86	0.49	-3.79	<0.001
Entry time	0.07	0.02	3.61	<0.001

[a] The ALAN-exposed group is used as reference for the group effect

[b] CDE is used as reference for the population effect

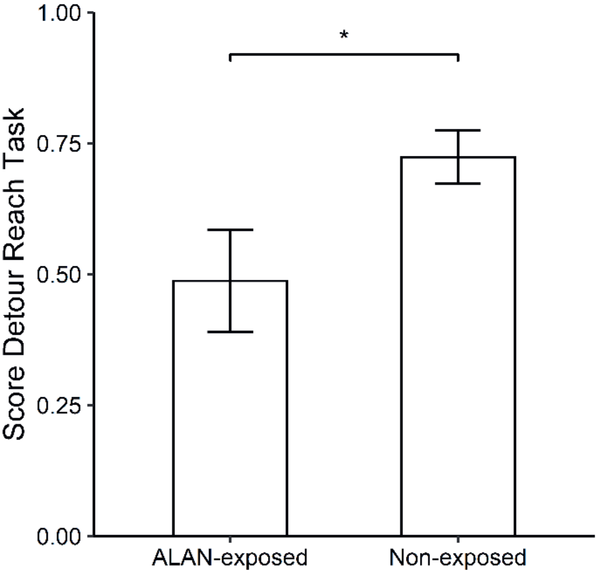

Figure 2: The score in the detour reach task (i.e. number of tests in which the worm was collected without pecking at the tube divided by the total number of tests in which the worm was collected) for the ALAN-exposed and the non-exposed group (± SE.; $n_{ALAN\text{-}exposed}$ = 16, $n_{non\text{-}exposed}$ = 19). For readability we here present proportions instead of success/fail data.

Per trial individuals pecked on average 7.2 times (i.e. considering all trials in which the bird collected the worm or pecked at the tube; ±s.d. = 9.0, min = 0, max = 29.9). The difference in the amount of pecks between individuals from the ALAN-exposed and the non-exposed group was marginally non-significant (W = 225, P = 0.08; $n_{\text{ALAN-exposed}}$ = 16, $n_{\text{non-exposed}}$ = 21; Figure 3). If only trials in which the individuals did peck at the tube were included (i.e. all successful trials were excluded), the average amount of pecking per trial was 16.5 (±s.d. = 15.2, min = 1, max = 61). If individuals pecked, then individuals from the ALAN-exposed group pecked more than the individuals from the non-exposed group (β ± SE = −1.00 ± 0.41, t = −2.45, P = 0.022, $n_{\text{ALAN-exposed}}$ = 13, $n_{\text{non-exposed}}$ = 16) and individuals with later entry times pecked less (β ± SE = −0.05 ± 0.02, t = −2.31, P = 0.029, n = 29).

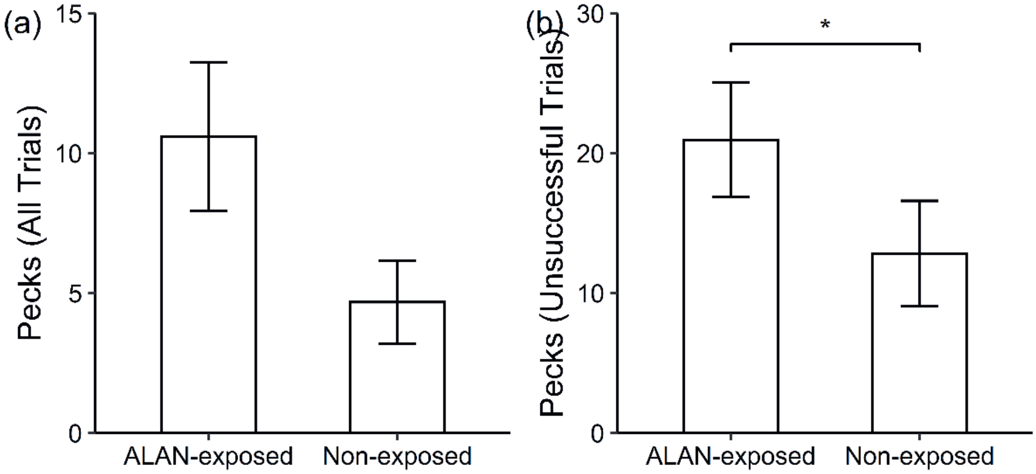

Figure 3: The average number of pecks at the tube during the detour reach task per individual (± SE) with (a) all successful trials (i.e. zero pecks) and all trials in which the bird pecked at the tube included ($n_{ALAN\text{-}exposed}$ = 16, $n_{non\text{-}exposed}$ = 21) and (b) only trials included in which the bird pecked at the tube ($n_{ALAN\text{-}exposed}$ = 13, $n_{non\text{-}exposed}$ = 16).

Vigilance

During the 20 s that were analysed, individuals scanned the environment on average 11.5 times (±s.d. = 6.2, min = 0, max = 27) and spent 32.0 % of the time on scanning (±s.d. = 16.5, min = 0, max = 69.1). Both the number of scans and the percentage of time spent on scanning were lower in the non-exposed group than in the ALAN-exposed group, although these effects were not statistically significant (number of scans: β ± SE = −0.26 ± 0.14, z = −1.84, P = 0.07; percentage of time spent on scanning: β ± SE = −6.69 ± 3.68, t = −1.82, P = 0.08; $n_{ALAN\text{-}exposed}$ = 24, $n_{non\text{-}exposed}$ = 26; Figure 4). The start time of the test affected both the number of scans and the percentage of time spent on scanning, with individuals tested later during the day being less vigilant (number of scans: β ± SE = −0.01 ± 0.003, z = −5.31, P < 0.001, n = 50; percentage of time spent on scanning: β ± SE = −0.39 ± 0.07, t = −5.62, P < 0.001, n = 50).

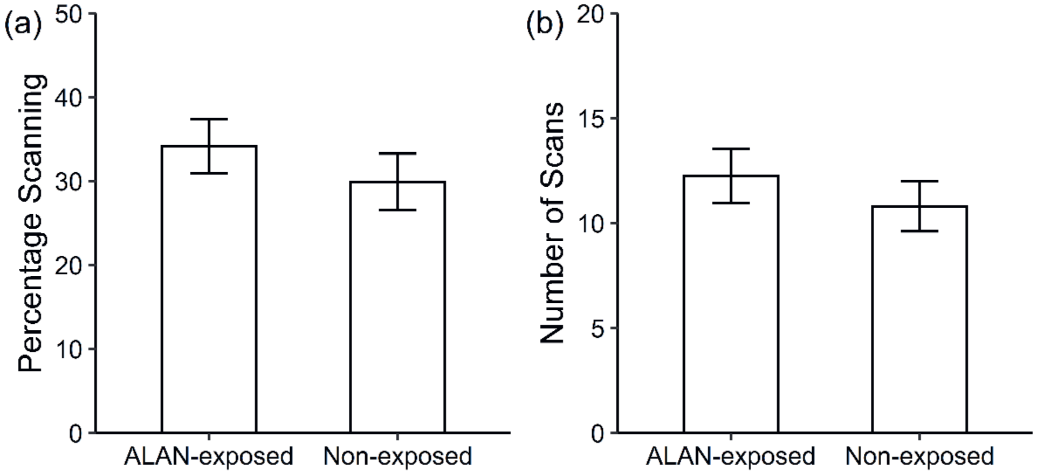

Figure 4: Vigilance behaviour in the ALAN-exposed and non-exposed group with (a) the percentage of time spent on scanning and (b) the number of scans (± SE; $n_{ALAN\text{-}exposed} = 24$, $n_{non\text{-}exposed} = 26$*).*

Discussion

Here, we investigated the effects of ALAN-exposure on cognitive performance in great tits. Our experiment was designed to mimic sleep deprivation by anthropogenic light pollution. We observed negative effects of ALAN-exposure on the performance in the detour reach task, which measures aspects of inhibitory control, but not on vigilance and exploratory behaviour. Furthermore, while we found substantial variation in cognitive performance among ALAN-exposed individuals, this variation was not related to an individual's habitual sleep rhythm as was hypothesised. Nevertheless, our expectation that ALAN-exposure affects cognitive performance in wild birds was supported by the results.

After exposure to ALAN, we found that individuals performed worse on the detour reach task compared to those not exposed to ALAN. It is generally assumed that the detour reach task measures differences between individuals in inhibitory control (Brandão et al., 2019; Coomes et al., 2022; Gomes et al., 2020; MacLean et al., 2014).

Hence, we can conclude that ALAN-exposure impaired inhibitory control, as hypothesised. Additionally, when great tits started to peck at the tube, ALAN-exposed individuals pecked more often than non-exposed individuals. Similar pecking behaviour in pheasant chicks has been linked to performance in a persistence test (van Horik et al., 2018), suggesting that ALAN-exposed individuals may exhibit greater persistence. While we only tested cognitive performance in captivity, recent research has demonstrated a relationship between performance on the detour reach task and a different inhibitory control test in the wild (Davidson et al., 2022), suggesting that results hold up in different contexts. Furthermore, inhibitory control is related to foraging flexibility (Coomes et al., 2022), dietary breadth (both among species and within species; MacLean et al., 2014; van Horik et al., 2018) and is linked to social complexity across species (Amici et al., 2008, 2018). Being able to inhibit prepotent responses may thus be relevant in a variety of contexts in wild animals.

In addition to inhibitory control, the detour reach task may be affected by other cognitive functions as well (reviewed by Kabadayi et al., 2018). Individual performance typically increases over trials (Isaksson et al., 2018; van Horik et al., 2020), which indicates that learning is involved in the detour reach task. However, here we found no signs of improvement over subsequent trials in both groups (i.e. no effect of trial and no interaction effect between group and trial; Table S10) and the differences we observed between both groups were likely not affected by learning. Furthermore, since we used wild individuals, they had to be returned to their territories in the early afternoon after testing, to ensure that they had enough time to forage before they returned to their nest box and to be able to defend the territory. Therefore, there were time constraints, and we decided not to include a training phase in our experiment, as is typically done in detour reach tasks (e.g. Davidson et al., 2022; Isaksson et al., 2018; Troisi et al., 2021). During the training phase individuals are supposed to learn to detour around an opaque object, i.e. the correct solution to the task, by repeating the tests several times. Since in our experiment great tits did not learn the solution to the detour reach task during a training phase, problem solving ability may have contributed to the observed differences between the experimental groups in our study (but see Yorzinski et al., 2017). However, the average success rate we observed in this study was comparable to success rates in other studies on wild great tits that did include a training phase (Coomes et al., 2022; Isaksson et al., 2018). Therefore it is not likely that problem solving caused the difference we observed between the ALAN-exposed and the non-exposed group. As learning may affect results from a detour reach task, this test could only be performed once for each individual and natural variation among individuals in their performance (i.e. pre-treatment) could not be assessed. In order to reduce the risk that the initial inhibitory control performance differed between our experimental groups we alternated between nights with and without ALAN-exposure (see Table 1).

During the detour reach task we found substantial differences among individuals from the ALAN-exposed group in their cognitive performance, which might indicate that there were either intrinsic cognitive differences among individuals, or that individuals differed in how much they were affected by ALAN-exposure. However, we did not find any relationship between the effect of ALAN-exposure on cognitive performance and the natural sleep behaviour in great tits, as we derived from recent studies in humans showing that the reduction of inhibitory control after sleep was related to an individual's habitual sleep rhythm and/or chronotypes (Barclay & Myachykov, 2017; Demos et al., 2016; J. Song et al., 2019). Since, unlike humans, great tits are tied to the daylight period for their activity (Meijdam, Müller, Thys, et al., 2022), there may be smaller variation in sleep duration and chronotype compared to humans. The among individual differences could be too small to detect in the effect of ALAN-exposure on cognitive performance. Furthermore, some individuals may be more sensitive to light than others (Raap, Pinxten, et al., 2016), which may have resulted in differences among individuals from the ALAN-exposed group in how much their sleep was restricted by the ALAN treatment. Thus, individual differences in the amount of sleep deprivation may also explain the variation in performance on the detour reach task.

While the natural sleep behaviour did not influence the impact of ALAN-exposure, we did find an effect of entry time into the nest box on cognitive performance. Individuals with later entry times under natural circumstances were more successful during the detour reach task and pecked less against the tube prior to accessing the prey. Interestingly, a meta study in humans showed a similar effect, with eveningness (i.e. a late chronotype) being related to higher cognitive capacities (Preckel et al., 2011), although the underlying mechanisms are still unclear. As we took the birds from their nest box in the evening, individuals with later entry times were possibly less disturbed during the experiment than individuals that were already sleeping for longer, as they may have been still awake or in an earlier stage of sleep (Vorster & Born, 2015).

Although we found that ALAN-exposure had an effect on inhibitory control, we did not observe an effect on vigilance behaviour in great tits. Vigilance is a highly plastic trait in birds that can be influenced by factors such as age, social environment, context (Mettke-Hofmann, 2022) and noise pollution (Klett-Mingo et al., 2016). Differences among individuals in their cognitive and sensory abilities may also impact vigilance (Tätte et al., 2019), and these may be impaired by sleep deprivation. Although we observed a marginal increase in scanning behaviour in the ALAN-exposed group compared to the non-exposed group, this difference was not statistically significant. This was unexpected, as research on vigilance performance after sleep deprivation in humans has shown that vigilant attention is typically reduced. However, a common method of measuring vigilant attention in humans is through the use of a psychomotor vigilance test, which assesses reaction times to a stimulus that are known to increase after sleep deprivation (reviewed in Hudson et al., 2020 and Lim & Dinges, 2008). The vigilance test we used in the great tits measured the amount of time spent scanning the environment and the number of scans, rather than reaction times, which could explain why we did not observe a reduction in vigilant behaviour. If sleep deprivation impairs the processing of visual information in birds, as it does in humans (Chee, 2015), then reaction times to visual predator signals may increase following exposure to ALAN, which could ultimately affect survival.

Here, we aimed to induce sleep deprivation by exposure to ALAN. Despite our efforts to film the birds' behaviour we were unable to precisely quantify the amount of sleep deprivation, as our camera could only cover one half of the cage. Nevertheless, we attempted to use the information we could obtain from the videos, by monitoring the birds' response to the lights turning on. In 87.5 % of the cases the birds were observed moving around when the lights were turned on, while birds not exposed to ALAN were often not visible or not moving in the footage. However, it is impossible to determine whether these non-moving birds were sleeping, as posture alone is not always an accurate indicator of sleep (Aulsebrook et al., 2016). While we believe that the ALAN-exposed individuals were more sleep deprived than the non-exposed individuals, based on their movements and previous studies indicating ALAN exposure leads to sleep deprivation (Aulsebrook, Connelly, et al., 2020; Aulsebrook, Lesku, et al., 2020; Raap et al., 2015; Raap, Pinxten, et al., 2016; Yorzinski et al., 2015), we cannot be entirely certain.

Sleep deprivation by exposure to ALAN may have consequences that could have indirectly affected cognitive performance. For example, it could impact overall activity (de Jong et al., 2016), feeding motivation (Hanlon et al., 2005) and stress levels (Ouyang et al., 2015). However, we did not observe any differences in exploratory or feeding behaviour between the ALAN-exposed and the non-exposed group, making it unlikely that these factors affected cognitive performance (see also Gomes et al., 2020). Furthermore, exposure to ALAN can also affect the circadian rhythm (Dominoni, 2015). In corvids exposure to dim light at night reduced the secretion of melatonin, a hormone involved in regulating the circadian rhythm, and led to cognitive impairment (Buniyaadi et al., 2022). Administering melatonin prevented this deterioration. Since sleep homeostasis and the circadian rhythm are closely linked, it is challenging to differentiate between their effects.

Conclusions

Our study reveals that intermittent exposure to ALAN led to a decline in inhibitory control in wild great tits, comparable to the effects of sleep deprivation in humans. This suggests that the relationship between sleep and cognition may be universal and that anthropogenic stressors like ALAN and noise pollution can negatively impact cognitive performance in wild birds. Although hole-nesting species such as the great tit may experience relatively minor effects of ALAN due to the shielding effect of nest boxes, especially at lower light intensities (Raap et al., 2018), not all individuals sleep inside these boxes throughout the year, leaving them vulnerable to ALAN exposure during specific periods. For instance, during the breeding season, only female great tits typically sleep inside the nest boxes, whereas males may face greater exposure (personal observation). This could have serious implications for fitness, as impaired cognitive performance during this critical period may be detrimental. Although anthropogenic stressors in the wild may disturb sleep to a lesser extent than in our experiment, i.e. a lower dose of ALAN (A. S. Grunst et al., 2023), they are often combined with other stressors, and can be present for much longer. This prolonged exposure could lead to chronic sleep deprivation (Aulsebrook, Lesku, et al., 2020). Therefore, future studies should investigate the possible consequences of long-term sleep deprivation due to anthropogenic stressors on cognitive performance in wild animals, and whether individuals can habituate to sleeping in the presence of anthropogenic stressors and to what extent.

Supplementary material

The supplementary information accompanying this chapter can be found on pages 180 - 191 of this thesis and is available online at DOI:10.1016/j.scitotenv.2023.163765

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Marcel Eens: Conceptualization, Methodology, Writing – review & editing.

Wendt Müller: Conceptualization, Methodology, Writing – review & editing.

Acknowledgements

We thank Peter Scheys, Geert Eens and Clara Garcia-Co for their field assistance. This work was supported by the University of Antwerp and FWO Flanders (FWO projects ID: G052117N to ME and Rianne Pinxten). We thank both for their support.

Chapter 5

Female chronotype and aggression covary on different hierarchical levels in a songbird

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Published in Animal Behaviour, 2024, 210, 75-83

https://doi.org/10.1016/j.anbehav.2024.02.001

Abstract

Individual variation in the timing of activities is increasingly being reported for a wide variety of species, often measured as the timing of activity onset in the morning. However, so far, the adaptive significance of consistent variation in temporal phenotypes (i.e. the chronotype) remains largely elusive. Potentially, differences in timing of activities may arise as a result of competition among individuals for resources. Less aggressive individuals may try to avoid competition by becoming active earlier during the day when other individuals are still inactive, leading to a positive correlation between chronotype and aggressive personality type (i.e. a behavioural syndrome). To investigate this, we assessed the chronotype of female great tits, *Parus major*, by measuring emergence time from the nest box in the morning and experimentally tested their levels of same-sex aggression through simulated territorial intrusion tests. Contradicting our initial hypothesis, consistently more aggressive females became active earlier during the day compared to less aggressive females, which could be caused by shared underlying mechanisms, like pleiotropic effects of sex hormones or gene pleiotropy, which potentially impose constraints on the independent evolution of both traits. Surprisingly, on the within-individual level we found an opposing correlation between emergence time and aggression. Our findings highlight the need for further investigations into the interplay between chronotype and aggression that take the underlying mechanisms into account in order to understand the adaptive significance of this trait association.

Introduction

In both humans and nonhuman animals, behaviour and physiology often show rhythmicity that is aligned with the day and night cycle. For example, sleep – wake cycles and body temperature fluctuations follow patterns of approximately 24 h (e.g. Aschoff, 1967; Moore, 2007; Refinetti & Menaker, 1992; Strauß et al., 2022). This rhythmicity is maintained by the circadian clock, a biochemical timekeeping system that is sensitive to light. In birds, differences between individuals have been observed in the timing of the circadian clock (Helm & Visser, 2010; Lehmann et al., 2012; Tomotani et al., 2023). Such differences become apparent when studying the wake-up time in the morning or the timing of the onset of sleep in the evening. Consistency in the timing of activities is typically referred to as an individual's chronotype. Although an increasing number of studies have demonstrated the existence of chronotypes in multiple species (Alós et al., 2017; Chmura et al., 2020; Dominoni, 2013; Graham et al., 2017; M. L. Grunst et al., 2021; Maury et al., 2020; Meijdam, Müller, & Eens, 2022; Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015), its adaptive significance and how this variation is maintained in the population remain largely unknown.

Behavioural traits that consistently vary among individuals across contexts and over time are often considered as personality traits (Réale et al., 2007). The existence of consistent differences between individuals implies that behavioural plasticity is limited and that some individuals may be coping better in certain environments than others (Dingemanse et al., 2012). Natural and sexual selection may thus act upon such personality traits, and in order to evolve they should be genetically underpinned (Dochtermann et al., 2015, 2019). Although daily activity patterns are often highly repeatable across time and context and the circadian period length, a key regulator of the chronotype (Tomotani et al., 2023), is highly heritable ($h^2 = 0.86$, SD = 0.24; Helm & Visser, 2010), few studies have considered it as a personality trait in nonhuman animals (Randler, 2014). Further, individual variation in chronotype might come with fitness consequences. For example, starting activity early could improve feeding opportunities, while it might increase predation risk by (nocturnal) predators (Helm et al., 2017). The timing of sexual displays in turn may affect mating success (Hau et al., 2017). Indeed, in male eastern kingbirds, *Tyrannus tyrannus*, and blue tits, *Cyanistes caeruleus*, individuals that start dawn song earlier in the morning are more likely to gain extrapair paternity (Dolan et al., 2007; Poesel et al., 2006; Schlicht et al., 2023).

However, in blue tits, experimentally advancing the dawn song did not increase extrapair success in males (Santema & Kempenaers, 2023) and chronotype was not related to extrapair success in females (Schlicht et al., 2014). Chronotype did also not affect the female's reproductive success in blue and great tits, *Parus major* (Steinmeyer et al., 2013; Womack, 2020; but see Womack et al., 2023a). Thus, the current state of knowledge is still inconclusive, especially in females.

Importantly, selection may act on suites of correlated behaviours simultaneously (Bell, 2006; Sih et al., 2004). Such a correlation between two or more behavioural traits at the among-individual level is typically referred to as a behavioural syndrome (Sih et al., 2004). For chronotypes, behavioural syndromes are underexplored at least in nonhuman species (but see Alós et al., 2017; Amin et al., 2016). Behavioural syndromes can arise by multiple processes. One such process is described by the adaptive hypothesis, which states that correlations between behavioural traits emerge when the correlation itself is adaptive (Bell, 2005; Herczeg & Garamszegi, 2012). In other words, the combination of two or more traits is favoured by correlational selection (Bell, 2005). For example, in sticklebacks, *Gasterosteus aculeatus*, some individuals may opt for safety in numbers by schooling, which does not go well with aggressiveness towards conspecifics (Magurran & Seghers, 1991). Other individuals may inspect a predator to gain information (Bell & Sih, 2007), where aggressiveness may be beneficial, potentially leading to a behavioural syndrome between the antipredator strategy and aggression (see also Huntingford, 1976). However, behavioural syndromes do not need to be adaptive per se. From a mechanistic perspective they may also arise when different behaviours are controlled by the same gene(s) (i.e. gene pleiotropy), neurotransmitters or hormones. Such shared underlying drivers of behaviour may constrain the independent evolution of the traits involved, for example through antagonistic selection, so that the trait optima do not coincide. This hypothesis is referred to as the constraint hypothesis (Bell, 2005).

Finally, individual variation may also relate to interindividual differences in realized ecological niche dimensions (i.e. individual niche specialization; Bolnick et al., 2003). One of the main drivers of individual specialization is thought to be intraspecific competition, as it can favour niche width expansion or diversification (Dall et al., 2012; Svanbäck & Bolnick, 2006). Temporal segregation in daily activity patterns within species, which may result from differences between individuals in chronotype, could thus be related to competition (Alanärä et al., 2001; Howerton & Mench, 2014; D. B. Stone et al., 2019), and may be related to other personality traits, if they determine the competitive ability of the individual (Gharnit et al., 2020), leading to behavioural syndromes.

Competitive ability is, among others, related to aggression (Blanchard et al., 1988; O'Shea et al., 2017), which serves a critical function in resolving conflicts among conspecifics when they compete for resources like territories, partners or food sources (Pandolfi et al., 2021), and can be either defensive or offensive (Blanchard et al., 2003). Conflict resolution can involve signalling, such as through birdsong, or physical aggression (van Staaden et al., 2011), with the latter carrying the potential risk of injury. Individuals with low aggression levels could benefit from being active during periods when others are asleep, which enables them to obtain the necessary resources. In contrast, starting activity early may not be necessary to more aggressive individuals. Therefore, we predict a positive correlation between chronotype and aggression, as has been reported in pearly razor fish, *Xyrichtys novacula* (Martorell-Barceló et al., 2023).

We used female great tits to test these predictions, as previous studies in our population have shown both short- and long-term repeatable variation in aggression (Thys et al., 2021) and emergence time (i.e. the timing of activity onset in the morning; Meijdam, Müller, & Eens, 2022), which is a prerequisite for the existence of behavioural syndromes. Moreover, aggression in great tits is related to exploration and risk taking (Carere et al., 2005; Hollander et al., 2008; Thys et al., 2017), and all three behavioural traits have been related to dominance in foraging contexts and higher foraging flexibility (Bibi et al., 2019; Coomes et al., 2022; Dingemanse & de Goede, 2004; Verbeek et al., 1996), indicating that more aggressive great tits are likely to be better competitors. To test for a behavioural syndrome between chronotype and aggression, we measured emergence time from the nest box in the morning as a proxy for chronotype, and determined aggression by experimentally simulating territorial intrusions, both during the egg-laying period of the breeding season. As phenotypic correlations between two (or more) behavioural traits may suggest the presence of a behavioural syndrome, but could also arise from correlated plasticity, we partitioned the phenotypic correlations at two levels: the among-individual level, which corresponds to a potential behavioural syndrome, and the within-individual level, which is indicative of correlated plasticity (Dingemanse, Dochtermann, et al., 2010).

Methods

Study Population

All data were collected in a suburban nest box population of great tits (±170 nest boxes), in Wilrijk (Antwerp), Belgium (51°09′46.1″N, 4°24′13.3″E) during the breeding season (March – May) of 2018 and 2019 as part of two previous studies (Meijdam, Müller, & Eens, 2022; Thys et al., 2021). All nest boxes were monitored for nest building, egg laying and incubation. During previous breeding seasons and during roosting in winter individual birds were equipped with unique combinations of rings around their tarsi, enabling individual recognition. Each bird received a metal ring and three colour rings, one of which contained a PIT-tag (passive integrated transponder; internal diameter 2.6 mm, length 8.0 mm, EM4102, 125 KHz, Eccel Technology Ltd, Aylesbury, U.K.). Age (yearling versus older) and sex were determined based on plumage characteristics upon capture.

Ethical Note

This study was approved by the ethical committee of the University of Antwerp (ID numbers: 2016-87, 2018-50, 2017-23 and 2017-61) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complied with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licences for all authors and technicians. Handling time was minimized as much as possible. All other methods described below are non-invasive.

Emergence Time

During the breeding season, only females sleep inside the nest box. Female emergence times from the nest box were measured as a proxy for activity onset (see Meijdam, Müller, & Eens, 2022; Meijdam, Müller, Thys, et al., 2022) during the egg-laying period of the breeding season, since both aggression and emergence time could simultaneously be measured during this period. Emergence times reported here were mainly determined using SongMeters (682 data points on 209 females, SongMeterTM SM2+; Wildlife Acoustics, Inc., Maynard, MA, U.S.A.), but during the peak of the 2018 breeding season the number of females that had to be measured on the same day was higher than the number of SongMeters available, so we additionally used radiofrequency identification (RFID) loggers (14 data points on 10 females, EM4102 data logger, Eccel Technology Ltd, Aylesbury, U.K.; Iserbyt et al., 2018) and infrared sensitive cameras (one data point on one female, Pakatak PAK-MIR5, Essex, U.K., Grunst et al., 2022). Across the breeding season emergence times were often measured with both RFID loggers and SongMeters simultaneously. On average they differed by 1.5 ± 0.4 min (mean ± SE; N = 633 mornings recorded at 74 nest boxes, M. L. Grunst et al., 2024). To ascertain whether there was any bias in the results due to the different methodologies used, we performed our statistical analyses once with all data included and once with only SongMeter data included. Since both analyses gave similar results, we only present those of the analysis using all emergence time data.

SongMeters were placed on top of the nest box with one microphone inside and the other outside the nest box. Sound was recorded from 0400 hours to 0800 hours during the winter. After the clock changed to summertime sound was recorded from 0300 hours to 0800 hours. Avisoft SASLab Pro 5.2.14 (Avisoft Bioacoustics, Berlin, Germany) was used to visualize the sound profiles (Figure S1) and emergence time was determined by inspecting the sonogram and audio (Specht, 2002). When a female leaves the nest box in the morning the sound of the body moving through the nest box opening can be heard on the inside microphone, while the wing beats during take-off can be heard on both microphones (Halfwerk et al., 2011). The moment of take-off was used as the emergence time. Because of the order of sounds made by the female, it was easy to identify the direction of the movement. RFID loggers registered PIT-tagged individuals when they flew through the two antennas, which were placed around the nest box opening. Both the unique PIT-tag number and the time of leaving/entering the nest box were saved (for more details see Iserbyt et al., 2018). Infrared sensitive cameras were installed under the lid of the nest box, pointing downwards. The cameras started recording immediately after installation at least 2 h before sunset and were switched off on collection the next morning at least 2 h after sunrise (Raap et al., 2015). Within years each female was measured on 1–7 consecutive mornings during the egg-laying period.

Aggression

Aggression tests were performed on day 2 and 5 (or day 3 and/or 6 if the female was not present on the first time point) of the egg-laying period in both years, with day 1 referring to the day the first egg was laid (Thys et al., 2017). A territorial intrusion was simulated by placing a taxidermic mount of a female great tit inside a wire mesh cage (decoy; $N_{decoy} = 6$) on top of the focal female's nest box, following Thys et al., 2017).

From the moment the focal female was present within a radius of 15 m around the nest box, her behaviour was observed ($N_{observer}$ = 6) for 5 min from approximately 15 m away. For the focal female, the following aggression parameters were scored: the minimal distance to the decoy (approach distance, m), the number of alarm calls produced, the number of attacks towards the decoy and the time spent on the wire mesh cage (time on decoy, s). All tests were performed between 0730 hours and 1200 hours in the morning.

Statistical Analyses

In total, data on emergence time were collected for 207 females. Data of 15 females were removed from the data set since their age remained unknown due to nest failure before they could be captured. The final data set contained 43 females for which emergence times were measured in both years and 149 females for which emergence times were measured in 1 year (average number of measurements within years = 3.71, SD = 0.98). For 181 females we also obtained data on aggression (N_{test} = 401), with 39 females measured in both years and 142 females measured in 1 year (average number of measurements within years = 1.72, SD = 0.45). All statistical analyses were performed in R 4.1.3 (R Core Team, 2013). The MCMCglmm package (version 2.35) was used throughout to fit multivariate mixed models (Hadfield, 2010). Response variables were scaled to unit variance prior to all analyses.

We performed two consecutive analyses. First, we explored the among- and within-individual (co)variation between the different aggression parameters observed during territorial intrusion. To this end, we constructed a multivariate mixed model (Araya-Ajoy & Dingemanse, 2014; Dingemanse & Dochtermann, 2013; Houslay & Wilson, 2017) which included the approach distance (multiplied by −1), the number of calls, the number of attacks and the time on the decoy as response variables of which the latter three were square root transformed. Based on previous findings (Thys et al., 2021), year (2018 versus 2019) and age (yearling versus older) were included as fixed effects.

Female identity (N = 181) was included as a random effect, which enabled us to partition behavioural (co)variation into its among- and within-individual components (Dingemanse & Dochtermann, 2013). We did not include decoy or observer identity as random effects since our previous work has shown they are of no or minor importance in explaining variation in aggression parameters (see Thys et al., 2020, 2021). From this model, the repeatability of each separate aggression parameter was calculated as the among-individual variance divided by the total phenotypic variance (i.e. the sum of among- and within-individual variance; Nakagawa & Schielzeth, 2010). Among- and within-individual covariances between the different aggression parameters were converted into correlations by dividing the respective covariance by the square root of the product of the respective variances, thereby allowing easier interpretation.

Second, a multivariate mixed model was constructed to investigate the among- and within-individual (co)variation between emergence time from the nest box and aggression. Results of our first multivariate mixed model revealed that time on the decoy during territorial intrusion covaried with the number of attacks on both the among- and within-individual levels (see Results and Table 1) so we opted to use time on the decoy in further analyses as an operational measure of aggression (see also Araya-Ajoy & Dingemanse, 2014). Hence, we constructed a bivariate mixed model with time on the decoy (square root transformed) and emergence time from the nest box as response variables. As we had more measurements for emergence time than for time on decoy, the measurements were not always matched. For both traits, we included year (2018 versus 2019), age (yearling versus older) and a polynomial date (mean-centred within years) effect up to the second order as fixed effects (Meijdam, Müller, & Eens, 2022; Thys et al., 2021). For emergence time, we also included the temperature (T°) at sunrise (mean-centred within years; Schlicht & Kempenaers, 2020). Female identity (N = 181) was included as a random effect and repeatability and among- and within-individual correlations between emergence time and time on the decoy were calculated as described above.

Table 1: Correlations (with 95% credible intervals) between the aggression parameters on the among-individual (above the diagonal) and within-individual level (below the diagonal).

	Approach distance	No. calls	No. attacks	Time decoy
Approach distance	-	- 0.12 (- 0.55; 0.35)	0.18 (-0.52; 0.72)	0.08 (-0.65; 0.65)
No. calls	**-0.16** **(-0.29; -0.04)**	-	-0.17 (-0.54; 0.23)	-0.17 (-0.52; 0.22)
No. attacks	**0.19** **(0.06; 0.32)**	-0.09 (-0.24; 0.05)	-	**0.85** **(0.63; 0.95)**
Time decoy	**0.38** **(0.26; 0.50)**	**-0.22** **(-0.36; -0.08)**	**0.79** **(0.73; 0.84)**	-

All multivariate mixed models were run with Gaussian error distributions. We set the number of iterations at 420 000, the burn-in phase at 20 000 and the thinning interval at 200. The results presented are from models with a noninformative parameter expanded prior (see supplementary material for prior specification). The use of alternative prior specifications (i.e. inverse Wishart and inverse gamma) gave qualitatively similar results. Traces of posterior distributions were checked visually and autocorrelation between successively stored iterations was less than 0.1 in all cases (Hadfield, 2010). Model convergence and mixing were assessed using Gelman Rubin statistics between chains (i.e. the potential scale reduction factor was <1.1 in all cases; Gelman & Rubin, 1992). Results presented are posterior mean estimates with associated 95% credible intervals (CrI), unless stated otherwise. Fixed-effect and correlation estimates were considered to find strong support if 95% CrI did not overlap with zero.

Results

Sources of (Co)variation in Aggression Parameters

Repeatability of aggression parameters was low to moderate ($R_{\text{approach distance}}$ = 0.14 [0; 0.26], $R_{\text{number of calls}}$ = 0.33 [0.20; 0.45], $R_{\text{number of attacks}}$ = 0.28 [0.12; 0.46], $R_{\text{time decoy}}$ = 0.26 [0.11; 0.41]; see Table S1 for descriptive statistics on aggression parameters). On the among-individual level, we only found support for a correlation between the number of attacks and the time on the decoy (Table 1). On the within-individual level, all variables were correlated except for the number of attacks and the number of calls.

Emergence Time and Aggression

Repeatability of emergence time was high (R = 0.61 [0.54; 0.68]; see Table S1 for descriptive statistics on emergence time). Emergence time was affected by date. As the breeding season progressed, females emerged later from their nest box relative to sunrise (Table 2). Time on the decoy was affected by age, with yearlings spending on average more time on the decoy than older females (Table 2). In 2019, females spent on average less time on the decoy than in 2018.

Table 2: Results from a bivariate mixed model with emergence time from the nest box (in minutes relative to sunrise) and time on the decoy (seconds) as response variables. Estimates of fixed (β) and random (σ²) components are shown with 95% credibility intervals (CrI) and fixed effects that found strong support are given in bold.

	Emergence time	Time decoy
Fixed effects	β (95% CrI)	β (95% CrI)
Intercept	**0.18 (0.05; 0.33)**	**0.46 (0.30; 0.64)**
Year[a]	-0.15 (-0.29; 0.01)	**-0.33 (-0.52; -0.16)**
Age[b]	-0.19 (-0.36; 0.00)	**-0.68 (-0.90; -0.47)**
Date[c]	**3.61 (0.08; 7.14)**	-3.11 (-7.47; 1.48)
Date 2[c]	**5.13 (2.01; 8.07)**	-3.96 (-8.81; 0.92)
T° at sunrise[d]	-0.01 (-0.03; 0.02)	-
Random effects	σ² (95% CrI)	σ² (95% CrI)
FemaleID	0.61 (0.47; 0.76)	0.31 (0.16; 0.48)
Residual	0.38 (0.34; 0.43)	0.65 (0.52; 0.78)
$\mathrm{Cov}_{\text{among individuals}}$	-0.21 (-0.33; -0.10)	-
$\mathrm{Cov}_{\text{within individuals}}$	0.17 (0.07; 0.27)	-
$\mathrm{Cor}_{\text{among individuals}}$	-0.48 (-0.72; -0.25)	-
$\mathrm{Cor}_{\text{within individuals}}$	0.35 (0.15; 0.53)	-
Repeatability	0.61 (0.54; 0.68)	0.32 (0.19; 0.47)

[a] 2018 is used as reference year

[b] Yearling is used as reference age

[c] Date of measurement mean centred within years

[d] Temperature at sunrise mean centred within years

We found strong support for a negative among-individual correlation between emergence time from the nest box and time on the decoy. Females that consistently emerged earlier from the nest box spent consistently more time on the decoy compared to females that emerged later (r = −0.48 (−0.72; −0.25); Table 2, Figure 1a). On the within-individual level there was strong support for a correlation between emergence time and time on the decoy in the opposite direction. Specifically, an individual female was more aggressive when she emerged later, relative to her average emergence time, compared to when she emerged earlier (r = 0.35 (0.15; 0.53); Table 2, Figure 1b).

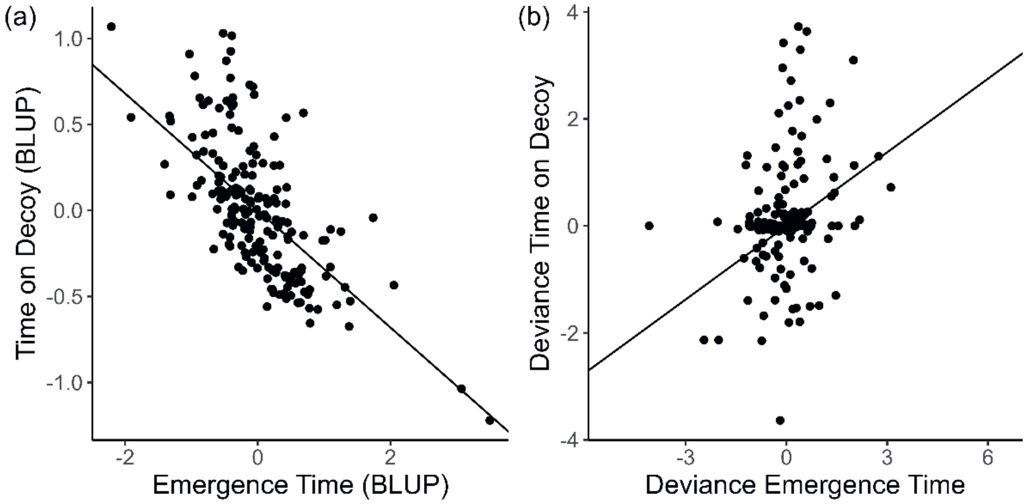

Figure 1: Correlation between emergence time from the nest box and time spent on the decoy during a simulated territorial intrusion in female great tits (N = 181) at the among-individual level (A) and the within-individual level (B). BLUPs were extracted from a bivariate mixed model (Table 2) and used here for illustrative purposes only (Houslay & Wilson, 2017). The within-individual effect was visualised by mean-centring the data within individuals for both emergence time and time on decoy.

Discussion

To better understand the costs and benefits of among-individual variation in chronotypes we investigated whether chronotype was part of a behavioural syndrome. More specifically, we studied whether chronotype was related to aggressive personality type in female great tits, as we expected that less aggressive females would benefit from an earlier onset of activity to avoid intraspecific competition. Contrary to our hypothesis, we found that females with an earlier onset of activity were consistently more aggressive. Intriguingly, we also found evidence for correlated plasticity between these two traits, that is, a correlation at the within-individual level. Females were more aggressive when they emerged later relative to their average emergence time, and less aggressive when they emerged earlier. Below we discuss the possible causes of our findings from both a functional and mechanistic perspective.

During the territorial intrusion test we scored the approach distance, number of calls, time spent on the decoy and number of attacks. We used time on the decoy as a proxy for aggression, which covaried positively both among and within individuals with attacks, hence reflecting confrontational aggression. Notably, the correlations between the number of calls and the other behaviours were negative, both at the among- and within-individual level. Calling from a distance without physical contact might represent an alternative strategy to direct aggression in females, that is, nonconfrontational aggression (Boiten et al., 2023; Thys et al., 2017). However, this contradicts findings in male great tits, where the number of calls was positively correlated with the number of attacks and approach distance (Araya-Ajoy & Dingemanse, 2014; Samplonius, 2019). Yet, in blue tits, clear differences between same-sex aggression in males and females have been observed: females approached a female decoy closer, called much less, spent more time on the decoy and attacked more often than males when they were tested with a male decoy. Potentially, aggression in males and females may not be directly comparable due to their unequal investment in the nest (Boiten et al., 2023).

Females with an early onset of activity spent on average more time on the decoy, which contradicted our hypothesis that less aggressive personality types may benefit more from being early compared to more aggressive females, as it allows the former to avoid competition in a foraging context. The negative correlation between chronotype and aggressive personality type could possibly be explained by the fact that aggression correlates with risk-taking behaviour (Barnett et al., 2012; Bell & Stamps, 2004; K. E. Cain et al., 2011; Huntingford, 1976). Starting activity early could in turn be more risky as it might lead to higher levels of predation, since vision in great tits is impaired if light conditions are not optimal (Kacelnik, 1979). The negative among-individual correlation between chronotype and aggression thus potentially reflects differences in the level of risk taking, representing a more extended behavioural syndrome.

On a mechanistic level, a negative relationship between chronotype and aggressive personality type could relate to differences between individuals in sex steroid concentrations or in the sensitivity to sex steroids (sensu the constraint hypothesis; Bell, 2005). Recent evidence has demonstrated that circadian rhythms are modulated by sex steroids and that the circadian clock has receptors for sex hormones (reviewed by Elderbrock et al., 2021). Notably, gonadotropin-releasing hormone-induced levels of oestradiol are related to chronotype in female dark-eyed juncos, *Junco hyemalis*, with individuals with higher peak levels of oestradiol showing earlier chronotypes (Graham et al., 2019). Sex steroids may also affect aggression, although the exact mechanisms underlying this relationship are still not fully understood (Hau et al., 2000; Heimovics et al., 2015, 2018; Quintana et al., 2021; Soma, 2006; Soma et al., 2008). Further, in the run-up to the breeding season, a period with great changes in sex steroid concentrations for both males and females, males tend to start activity progressively earlier in the morning than females (Schlicht & Kempenaers, 2020; Stuber, Dingemanse, et al., 2015), which may suggest that chronotype is regulated by sex hormones in male great tits too.

Additionally, genes that are involved in the circadian clock may have pleiotropic effects. For example, the *Clock* gene, which plays an important role in determining the circadian rhythm, is known to affect behaviour in mice, *Mus musculus* (Easton et al., 2003). Furthermore, mice that were selected for higher activity during the night were more aggressive and had altered expressions of genes that are involved in the circadian rhythm (i.e. *Clock*, *Per1* and *Per2*; Kerman et al., 2012). The circadian rhythm of aggressive mice is also less sensitive to light and the period length in the absence of light is closer to 24 h than that of less aggressive mice (Benus et al., 1988). Knocking out a circadian nuclear receptor (*Rev-Erbα*) increased aggression in mice (Chung et al., 2014) and aggression levels showed circadian rhythms (Todd et al., 2018). Pleiotropic effects of sex steroids and gene expression could thus be promising targets for future research aiming to unravel the mechanistic underpinnings of the correlation between chronotype and aggressive personality type. Uncovering the proximate mechanisms driving this behavioural syndrome could, in turn, provide a more profound understanding of the functional consequences of chronotypes.

At the within-individual level we, however, found a positive correlation between emergence time and aggression, that is, evidence for correlated behavioural plasticity (Pigliucci, 2003; Sheehy & Laskowski, 2023). It has been argued that suites of plastic behaviours may covary in response to a single factor or to a set of environmental variables that covaries itself (Sheehy & Laskowski, 2023). In our study system, one may argue that increased food availability may on the one hand allow individuals to spend more time in the nest box, thereby avoiding the high-risk twilight conditions, as they might need less time for foraging when conditions are favourable. On the other hand, patches with temporarily high food availability likely attract more conspecifics, and high social densities are known to increase aggression levels (Araya-Ajoy & Dingemanse, 2017; Quque et al., 2022; Yoon et al., 2012). Potentially, great tit females in our study plastically responded to two covarying environmental variables, that is, food conditions and social density, with an orchestrated short-term change in both aggression and onset of activity. Such correlated behavioural plasticity in suites of behaviours is thought to be particularly beneficial in changing environments, but empirical evidence on the adaptive significance of these correlations is largely lacking. However, as behavioural traits are notoriously plastic, it will be essential to gain a better understanding of correlated behavioural plasticity to understand among-individual variation in behaviour. From a mechanistic viewpoint, pleiotropic effects, of which we argued above that they could give rise to behavioural syndromes, are also likely to generate correlated plasticity (Dochtermann, 2023; Ellers & Liefting, 2015).

Our finding that the among-individual and within-individual correlations between emergence time and aggression are opposite could stem from their potential association with trade-offs in investment (Dochtermann, 2023; Downs & Dochtermann, 2014; Van De Pol & Wright, 2008). That is, within individuals, the allocation of available resources (such as energy) to one trait may come at the expense of another trait, but, among individuals, variation in individual quality or state may result in differences in overall resource acquisition (Laskowski et al., 2021). In the great tit, only high-quality females might have enough resources available to invest in both aggression and early activity. In turn, increased aggression and earlier activity might also enhance resource acquisition, potentially creating a positive feedback loop. At the within-individual level, the trade-offs could likely depend on the environmental conditions. During the egg-laying period, when emergence time and aggression were measured, resources are still limited, and energy demands are high, due to egg production. Trade-offs may therefore be more prominent when compared to less demanding or more favourable periods. These trade-offs could in principle generate opposing selection pressures, which can imply either that the rate of directional change may be low so that population (genetic) variation is maintained, or that it may require changes in the environment to resolve it. Yet, more research on the occurrence and significance of within-individual behavioural correlations is necessary to address this critical gap in our understanding. We currently know too little to provide conclusive answers.

Conclusion

Overall, our study provides evidence for the correlated expression of chronotype and aggression on the among-individual level, but whether this trait combination is adaptive and hence favoured by selection still needs to be shown. We found considerable variation among individuals in both behaviours, suggesting that the fitness consequences of this behavioural syndrome may be frequency dependent or could be related to fluctuating environmental factors, such as the food conditions and the social density. Considering the above-mentioned studies in mice that demonstrated a mechanistic link between circadian clock characteristics and aggression (i.e. altered gene expression and the effect of knockouts) it is plausible that the link between chronotype and aggression reflects an underlying shared mechanism, rather than being the result of correlational selection. Finally, when studying the functional consequences of variation in chronotypes and/or aggression, it is crucial to consider the proximate processes that could lead to their correlated expression at both the among- and within-individual levels, as it is possible that they may not be able to evolve independently, potentially leading to suboptimal trait expression.

Supplementary material

The supplementary information accompanying this chapter can be found on pages 192 - 193 of this thesis and is available online at DOI:10.1016/j.anbehav.2024.02.001

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Acknowledgements

We thank Peter Scheys and Geert Eens for their field assistance. This work was supported by the University of Antwerp and FWO Flanders (FWO projects ID: G0A3615N and G052117N to Marcel Eens and Rianne Pinxten). We thank both for their support.

Chapter 6

Female chronotype is not related to annual and life time reproductive success in a free-living songbird

Meijdam, M., Eens, M., & Müller, W.

Abstract

Circadian clocks play a crucial role in regulating the sleep-wake rhythm of organisms, aligning their activity with fluctuating environmental factors, such as light intensity. Still, significant and consistent inter-individual differences in the timing of activity, known as chronotypes, have been observed across various species, but whether this affects fitness is still unknown. While previous studies have primarily focussed on annual reproductive success, individual differences in survival and life time reproductive success were as yet disregarded. Here, we study the association between chronotype and annual reproductive success, life time reproductive success and longevity in free-living female great tits, using a long-term dataset of individually marked birds. Using the emergence time from the nest box in the morning during the egg laying period to determine chronotype, the number of eggs and fledglings produced by a female in a given year as proxies for annual reproductive success, the total number of eggs, fledglings and recruits produced as proxies for life time reproductive success and the age at death as a measure of longevity, we revealed no significant relationships between chronotype and reproductive success and survival. While our findings imply a lack of directional selection on chronotypes, we found neither evidence of stabilising nor disruptive selection. Overall, our results suggests that the existing variation in this trait is maintained in the population.

Introduction

Prominent abiotic and biotic factors in the environment, such as the light conditions, temperature, food availability and the presence or absence of predators follow a daily cycle (Helm & Womack, 2018). Biological clocks are fundamental to the synchronisation of behaviour, such as the timing of activity, and physiology with such cycles. They provide a self-sustaining circadian oscillation of about 24 hours at the molecular level that is sensitive to the environment. Biological clocks are ubiquitous, and are considered to be functionally adaptive, as they may enable organisms to optimise their use of the environment (Bloch et al., 2013; Yerushalmi & Green, 2009). Intriguingly, within species there are often remarkable differences between individuals in the behavioural output of the circadian clock, i.e. the timing of activity onset and offset (Alós et al., 2017; Chmura et al., 2020; Dominoni, 2013; Graham et al., 2017; Maury et al., 2020; Meijdam, Müller, & Eens, 2022; Schlicht & Kempenaers, 2020; Steinmeyer et al., 2010; Stuber, Dingemanse, et al., 2015). An individual's temporal phenotype is typically called chronotype. Individual differences in chronotype may be caused by variation in the endogenous circadian period (τ), which is the duration of one full cycle of the circadian rhythm in the absence of any time giving cues. τ often slightly deviates from 24 hours (Brown et al., 2008; Pfeffer et al., 2015; Ralph & Menaker, 1988; Wicht et al., 2014). Individuals with relatively short cycle lengths have earlier chronotypes. Such individuals generally time their activity earlier in the day, and vice versa.

An individual's chronotype can have important functional consequences in the wild (Hau et al., 2017; Helm et al., 2017). Starting activity earlier during the day relative to conspecifics may increase feeding opportunities, which could in turn increase survival and reproductive success (Helm et al., 2017; Helm & Womack, 2018). However, early rising may also come with certain costs. For example, early rising individuals may face greater predation risk due to limited vision, or they could incur energetic costs due to low temperatures in the morning (Helm & Womack, 2018). Furthermore, in many bird species courtship occurs during a specific daily time window, typically early in the day. These opportunities and risks must be weighed against each other. Potentially, a specific starting time of activity exists that optimises the balance of opportunities and risks and thus maximizes fitness. Individual variation in chronotypes may therefore be related to fitness.

So far, studies investigating the reproductive consequences of variation in chronotype in the wild have shown that the timing of the dawn song in male birds is related to extra pair paternity (Dolan et al., 2007; Greives et al., 2015; Poesel et al., 2006; Schlicht et al., 2023; Steinmeyer et al., 2013, but see Santema & Kempenaers, 2023). Furthermore, female European starlings (*Sturnus vulgaris*) that initiated a second brood had earlier activity onset during the chick rearing period (Maury et al., 2020). However, no other measures of annual reproductive success, like clutch size and number of fledglings, were affected by chronotype (Maury et al., 2020). In female blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) chronotype was not related to annual reproductive success (Steinmeyer et al., 2013; Womack, 2020), although a more recent study found that female great tits with earlier chronotypes had more fledglings (Womack et al., 2023a). Current empirical evidence on the reproductive consequences of chronotype, particularly in females, is thus mixed. Importantly, it has been neglected that fitness is not only affected by reproductive success within a given year, at least in more long-lived species, but also by survival and hence future breeding opportunities, which has not been taken into account thus far. Including survival in such analyses and studying life time reproductive success instead of annual reproductive success may hence be crucial to increase our understanding of the effect that chronotype may have on fitness (McCleery et al., 2004), and may reveal new insights on adaptive functions and individual variation.

In this study we investigated the consequences of differences in the average timing of activity onset in the morning (i.e. chronotype) for reproduction and survival in female great tits in a suburban population, using a large long-term dataset, for which we have shown both short- and long-term repeatable variation in the timing of activity onset (Meijdam, Müller, & Eens, 2022). Using such a long-term dataset enabled investigating correlations between the timing of activity onset and fitness both at the among- and within individual level. The timing of activity onset in the morning was determined by measuring the emergence time from the nest box. Furthermore, we did not only examine annual reproductive success (i.e. annual number of eggs and annual number of fledglings), but also life time reproductive success (i.e. life time number of eggs, life time number of fledglings, life time number of recruits). We used longevity (i.e. age at death) as a measure of survival.

Methods

This study was carried out in a suburban, nest box breeding great tit population in Antwerp, Belgium (51°09’46.1"N, 4°24’13.3"E), that has been monitored since 1995 (Dauwe et al., 1999). Each year (1995 – 2023), individuals were captured when roosting in the nest box in winter, during which their age (yearling or older) and sex were determined based on plumage characteristics. To facilitate individual recognition, individuals received a metal ring, two colour rings and a PIT-tag (passive integrated transponder) around their tarsi. Individuals not captured during winter were instead caught on the nest during the chick rearing phase of the breeding season, when the chicks were at least 8 days old. Chicks were ringed when between day 9 and 14 days old. We routinely determined clutch initiation dates, clutch sizes and the number of fledglings since 1997 (Dauwe et al., 1999; Raap et al., 2015; Van Duyse et al., 2005). In our population second clutches occur and clutch sizes and number of fledglings were determined as for the first clutch.

Ethical note

This study was approved by the ethical committee of the University of Antwerp (ID numbers: 2016-87, 2017-23, 2017-61 and 2018-50) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complies with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licenses for all authors and technicians. Handling time was minimized as much as possible. All other methods described above are non-invasive.

Emergence time

In 2018, 2019, 2020 and 2021, female activity onset in the morning was repeatedly recorded during the egg laying period, by measuring the emergence time from the nest box in the morning. We mainly used SongMeters (N = 1427; SongMeterTM SM2+; Wildlife Acoustics, Inc, U.S.), complemented by radio frequency identification (RFID) loggers (N = 57; EM4102 data logger, Eccel Technology Ltd, Aylesbury, U.K.) and cameras (N = 5; Pakatak PAK-MIR5, Essex, UK; Grunst et al., 2022) if insufficient SongMeters were available. SongMeters were positioned on top of the nest box, with one microphone inside and the other microphone outside the nest box. Recording sessions were conducted from 04:00 to 08:00 a.m. CET during the winter time period, and after the transition to summer time recordings took place from 03:00 to 08:00 a.m. CET. Upon the females' departure from the nest box in the morning the sound of their wings, their claws on the nest box (Halfwerk et al., 2011) and the female passing through the opening of the nest box can be heard. Emergence time was determined using Avisoft SASLab Pro 5.2.14 (Specht, 2002). RFID readers registered PIT tagged individuals upon passing through the two antennas positioned around the nest box opening. The PIT tag number and the corresponding entry and exit times were recorded (for more details see Iserbyt et al., 2018). Infrared sensitive cameras, mounted under the nest box lid and facing downward, started recording immediately after installation at least 2 h before sunset and were collected the following morning at least 2 h after sunrise (Raap et al., 2015). Throughout the 2018 breeding season, emergence times were often simultaneously measured with both RFID loggers and SongMeters. On average, there was a 1.5 ± 0.4 min difference between the two methods (mean ± standard error; N = 633 mornings recorded at 74 nest boxes, Grunst et al., 2024). We determined emergence times relative to sunrise (negative = before sunrise, positive = after sunrise), and hereafter, emergence time always concerns relative times. Temperature data was retrieved via: https://www.wunderground.com/history/daily/be/antwerp. In total we measured 1489 emergence times in 297 females, of which 47 individuals were removed from the dataset since their identity was unknown due to nest failure before identification, or because they were already older than one year upon first capture (N = 21) and so their age could not be accurately estimated (see Table 1 for sample sizes per year; sample size for analysis N = 250 females). Emergence times were determined in all 4 years for

7 individuals, in 3 years for 22 individuals, in 2 years for 64 individuals and in only 1 year for 157 individuals.

Table 1: Emergence time sample sizes per year after removal of 47 individuals with unknown identity and/or age.

Year	$N_{females}$	Mean number of measurements per female	S.D. of the number of measurements per female
2018	106	3.12	1.08
2019	113	2.96	1.09
2020	103	3.89	1.10
2021	57	4.42	1.05

Reproduction and survival

For all individuals whose emergence time was recorded, we assessed annual reproductive success, life time reproductive success and longevity. Annual reproductive success was determined by the annual number of eggs (i.e. the sum of the number of eggs in the first and second clutch in a given year) and the annual number of fledglings (i.e. the sum of the number of fledglings in the first and second clutch in a given year). Annual number of eggs was not known in one year for seven individuals and number of fledglings was not known in one year for ten individuals. Life time reproductive success was determined by the life time number of eggs, life time number of fledglings and life time number of recruits (i.e. offspring recruited into the breeding population). Longevity was determined by the age at death, defined as the age at which individuals were last breeding in the population. Individuals considered dead were also not observed outside of the breeding season. Some of these individuals may have dispersed out of the breeding population, but as the number of adult immigrants was relatively low (N = 21 on 297 females) and the number of breeding pairs in the population is stable, we expect this was only a minor proportion. We excluded 13 individuals from the dataset that were still alive at the moment of data analyses in autumn 2023 (i.e. last seen in the 2023 breeding season). For 11 females one year was missing in their reproductive data, so these were excluded from the life time reproductive success analyses (final samples size N = 226 females). Never more than one year was skipped.

Statistical analyses

All statistical analyses were performed in R 4.1.3 (R Core Team, 2013). The MCMCglmm package (version 2.35) was used throughout to fit multivariate mixed models (Hadfield, 2010). Emergence time was scaled to unit variance prior to all analyses. Reproductive success and longevity variables were divided by the population mean to obtain relative values. To study the covariation between the relative annual number of eggs and emergence time from the nest box we constructed a bivariate mixed model that included both as response variables (Houslay & Wilson, 2017; N = 243). Year (2018, 2019, 2020 or 2021) and age (in years; 1 to 6) were included as fixed effects for both variables (Table 2). For emergence time we included a polynomial date effect (mean-centred within years) up to the second order and the temperature at sunrise (mean-centred within years; Meijdam et al., 2024; Meijdam et al., 2022; Schlicht & Kempenaers, 2020). At the level of the relative annual number of eggs we included the clutch initiation date (mean-centred within years). Female identity was included as random effect, which enabled partitioning the (co)variance into the among- and within-individual components (Dingemanse & Dochtermann, 2013). The among- and within-individual covariance between relative annual number of eggs and emergence time were converted into correlations by dividing the respective covariance by the square root of the product of the respective variances. A second bivariate mixed model was constructed to study the covariance between emergence time and the relative annual number of fledglings (N = 240). This model included the same fixed and random effects as described above (Table 2). We repeated these analyses using only the number of eggs and fledglings from first clutches (for the results see Table S1 and S2).

We constructed four bivariate models to study the covariation between chronotype and life time reproductive success that included emergence time and either relative life time number of eggs, relative life time number of fledglings, relative life time number of recruits and relative age at death as response variables. These models included year (2018, 2019, 2020 or 2021), age (in years), a polynomial date effect (mean-centred within years) up to the second order and the temperature at sunrise (mean-centred within years) as fixed effects at the level of emergence time (Table 2). Again female identity was included as random effect. Within-individual variances were fixed at 0.0001 for all life time reproductive success parameters and age at death (see supplementary material for the details on prior specifications).

To examine whether stabilising or disruptive selection was acting on emergence time we determined whether individuals with emergence times closer to the population mean had higher reproductive success and longer longevity than individuals with more extreme emergence times. Therefore, for each observation, we calculated the absolute deviation from the population mean for emergence time. Then, we ran bivariate mixed models with the absolute deviation from the population mean for emergence time (square root transformed) and either annual number of eggs, annual number of fledglings, lifetime number of eggs, lifetime number of fledglings, lifetime number of recruits, or age at death as response variables. We included the same fixed and random effects as described above (Table 2).

All multivariate models were run with Gaussian error distributions. We set the number of iterations at 420 000, the burn-in phase at 20 000 and the thinning interval at 200. For the models including annual number of fledglings we increased the iterations to 550 000, the burn-in phase to 50 000 and the thinning interval to 250, due to convergence issues. For the model that included the absolute deviation from the population mean for emergence time and age at death as explanatory variables, we increased the number of iterations to 850 000, the burn-in phase to 50 000 and the thinning interval to 400. The results presented are from models with a non-informative parameter expanded prior (see supplementary material for prior specifications). The use of alternative prior specifications (i.e. Inverse Wishart and Inverse Gamma) gave qualitatively similar results. Traces of posterior distributions were checked visually and autocorrelation between successively stored iterations was less than 0.1 in all cases (Hadfield, 2010). Model convergence and mixing was assessed using Gelman Rubin statistics between chains (i.e. the potential scale reduction factor was <1.1 in all cases; Gelman & Rubin, 1992). Results presented are posterior mean estimates with associated 95% credible intervals (CrI), unless stated otherwise. Fixed-effects and correlation estimates were considered to find strong support if 95% CrI did not overlap with zero.

Table 2: Overview of fixed effects included in the different bivariate models.

Response variables	*Fixed effects* Year	Age	Date	$Date^2$	Temperature	Lay date
Emergence time (1) & number of eggs (annual) (2)	1 & 2	1 & 2	1	1	1	2
Emergence time (1) & number of fledglings (annual) (2)	1 & 2	1 & 2	1	1	1	2
Emergence time (1) & number of eggs (life time) (2)	1	1	1	1	1	-
Emergence time (1) & number of fledglings (life time) (2)	1	1	1	1	1	-
Emergence time (1) & number of recruits (life time) (2)	1	1	1	1	1	-
Emergence time (1) & age at death (2)	1	1	1	1	1	-
Emergence time (deviation from population mean) (1) & number of eggs (annual) (2)	1 & 2	1 & 2	1	1	1	2
Emergence time (deviation from population mean) (1) & number of fledglings (annual) (2)	1 & 2	1 & 2	1	1	1	2
Emergence time (deviation from population mean) (1) & number of eggs (life time) (2)	1	1	1	1	1	-
Emergence time (deviation from population mean) (1) & number of fledglings (life time) (2)	1	1	1	1	1	-
Emergence time (deviation from population mean) (1) & number of recruits (life time) (2)	1	1	1	1	1	-
Emergence time (deviation from population mean) (1) & age at death (2)	1	1	1	1	1	-

Results

Emergence time ranged from 127 minutes before sunrise up to 91 minutes after sunrise (mean ± S.D. = 8.45 ± 17.67, median = 7.31) and was significantly affected by date, yet in a non-linear way (Table 3). Across the breeding season females emerged progressively later from the nest box and this effect became stronger towards the end of the breeding season. As the season progressed, emergence times also started to deviate more from the population mean (Table 4). On colder mornings females emerged later from the nest box, but temperature at sunrise did not affect the deviation of the emergence time from the population mean (Table 4). There was variation among years in emergence times, with earlier emergence times in 2020 compared to 2018. In 2019 emergence times were closest to the population mean. Furthermore, emergence times advanced with age (Table 3), but age did not affect the deviation of the emergence time from the population mean (Table 4).

The annual number of eggs ranged from 3 up to 19 (mean ± S.D. = 9.38 ± 3.06, median = 9, N = 243) and the annual number of fledglings ranged from 0 up to 15 (mean ± S.D. = 6.61 ± 3.38, median = 7, N = 240). In 2019, the annual number of eggs was significantly higher than in 2018 (Table 3 and 4) and the number of fledglings was lower in 2020 and 2021 compared to 2018 (estimate_{2019} = 0.03 [-0.10, 0.15], estimate_{2020} = -0.30 [-0.44, -0.17], estimate_{2021} = -0.20 [-0.36, -0.04]). The annual number of eggs and fledglings was lower for later clutch initiation dates (Table 3 and 4; $\text{Estimate}_{\text{annual number of fledglings}}$ = -0.02 [-0.03, -0.01]). Finally, the annual number of eggs increased with age, while the annual number of fledglings did not (Table 3 and 4; $\text{Estimate}_{\text{annual number of fledglings}}$ = -0.002 [-0.057, 0.048]). When only first clutches were considered for analysis, the year and age effects on the annual number of eggs and the effect of clutch initiation date on the annual number of fledglings disappeared (Table S1).

Table 3: Results from a bivariate mixed model with emergence time from the nest box (in minutes relative to sunrise) and the relative annual number of eggs as response variables. Estimates of fixed (β) and random (σ²) components are shown with 95% credibility intervals (CrI) and fixed effects that found strong support are presented in bold.

	Emergence time	Relative annual number of eggs
Fixed effects		
Intercept	**0.353 (0.179, 0.495)**	**0.880 (0.797, 0.952)**
Date[b]	**9.968 (6.604, 13.451)**	-
Date 2[b]	**3.645 (0.858, 6.769)**	-
Temperature at sunrise[c]	**-0.031 (-0.046, -0.016)**	-
Year 2019[a]	-0.092 (-0.240, 0.061)	**0.112 (0.030, 0.189)**
Year 2020[a]	**-0.305 (-0.498, -0.132)**	0.033 (-0.052, 0.110)
Year 2021[a]	0.211 (-0.025, 0.470)	0.060 (-0.038, 0.173)
Age	**-0.126 (-0.203, -0.037)**	**0.034 (0.000, 0.069)**
Clutch initiation date[d]	-	**-0.017 (-0.022, -0.011)**
Random effects		
FemaleID	0.486 (0.386, 0.596)	0.019 (0.004, 0.036)
Residual	0.487 (0.444, 0.529)	0.079 (0.062, 0.097)

[a] 2018 is used as reference year

[b] Date of measurement mean centred within years

[c] Temperature at sunrise mean centred within years

[d] Clutch initiation date mean centred within years

Table 4: Results from a bivariate mixed model with the absolute deviation from the population mean for emergence time (in minutes relative to sunrise) and the relative annual number of eggs as response variables. Estimates of fixed (β) and random (σ^2) components are shown with 95% credibility intervals (CrI) and fixed effects that found strong support are presented in bold.

	Emergence time (deviation from population mean)	Relative annual number of eggs
Fixed effects		
Intercept	-0.04 (-0.20, 0.11)	**0.88 (0.80, 0.96)**
Date[b]	**4.23 (0.72, 7.66)**	-
Date 2[b]	1.59 (-1.54, 5.03)	-
Temperature at sunrise[c]	-0.01 (-0.02, 0.01)	-
Year 2019[a]	**-0.17 (-0.33, -0.01)**	**0.11 (0.03, 0.19)**
Year 2020[a]	0.10 (-0.07, 0.27)	0.03 (-0.05, 0.11)
Year 2021[a]	0.17 (-0.05, 0.39)	0.06 (-0.04, 0.17)
Age	0.01 (-0.06, 0.09)	**0.03 (0.00, 0.07)**
Clutch initiation date[d]	-	**-0.017 (-0.023, -0.012)**
Random effects		
FemaleID	0.34 (0.25, 0.43)	0.02 (0.00, 0.03)
Residual	0.68 (0.63, 0.75)	0.08 (0.06, 0.09)

[a] 2018 is used as reference year

[b] Date of measurement mean centred within years

[c] Temperature at sunrise mean centred within years

[d] Clutch initiation date mean centred within years

The life time number of eggs ranged from 4 up to 68 (mean ± S.D. = 19.29 ± 14.05, median = 13.5, N = 226), the life time number of fledglings from 0 up to 60 (mean ± S.D. = 13.03 ± 9.98, median = 9, N = 226), the life time number of recruits from 0 up to 10 (mean ± S.D. = 0.88 ± 1.38, median = 0, N = 226) and age at death from 1 up to 7 (mean ± S.D. = 2.07 ± 1.31, median = 2, N = 226). Emergence time was not significantly correlated with any of the annual or lifetime reproductive success parameters nor with longevity, either at the among- and within individual level. The absolute deviance of the emergence time from the population mean was also not associated with reproductive success or longevity (Table 6). Individuals with emergence times closer to the population mean were thus not more successful than individuals with more extreme emergence times. Only including first clutches for the annual number of eggs and the annual number of fledglings did not change these results (Table S2).

Table 6: Covariances and correlations (with 95% credible intervals) regarding emergence time from the nest box in the morning and the absolute deviation from the population mean for emergence time in relation to reproductive success and age at death at the among- and within-individual level.

Response variables	Covariance among individuals	Correlation among individuals	Covariance within individuals	Correlation within individuals
Emergence time & number of eggs (annual)	0.00 (-0.02, 0.02)	-0.01 (-0.32, 0.28)	0.00 (-0.03, 0.03)	0.00 (-0.11, 0.11)
Emergence time & number of fledglings (annual)	0.00 (-0.03, 0.04)	0.03 (-0.54, 0.78)	0.01 (-0.02, 0.05)	0.04 (-0.06, 0.15)
Emergence time & number of eggs (life time)	-0.06 (-0.15, 0.03)	-0.12 (-0.28, 0.05)	-	-
Emergence time & number of	-0.04 (-0.14, 0.05)	-0.07 (-0.24, 0.09)	-	-

fledglings (life time)				
Emergence time & number of recruits (life time)	-0.02 (-0.20, 0.17)	-0.01 (-0.17, 0.15)	-	-
Emergence time & age at death	-0.05 (-0.13, 0.02)	-0.13 (-0.30, 0.03)	-	-
Emergence time (deviation from population mean) & number of eggs (annual)	0.00 (-0.024, 0.025)	0.02 (-0.29, 0.40)	0.01 (-0.02, 0.03)	0.07 (-0.05, 0.20)
Emergence time (deviation from population mean) & number of fledglings (annual)	-0.02 (-0.05, 0.02)	-0.23 (-0.86, 0.34)	-0.01 (-0.05, 0.03)	-0.01 (-0.13, 0.09)
Emergence time (deviation from population mean) & number of eggs (life time)	-0.03 (-0.12, 0.05)	-0.06 (-0.23, 0.12)	-	-
Emergence time (deviation from population mean) & number of fledglings (life time)	-0.05 (-0.14, 0.034)	-0.10 (-0.28, 0.07)	-	-
Emergence time (deviation from population mean) & number of recruits (life time)	-0.12 (-0.29, 0.06)	-0.12 (-0.28, 0.05)	-	-
Emergence time (deviation from population mean) & age at death	-0.06 (-0.12, 0.02)	-0.15 (-0.32, 0.04)	-	-

Discussion

Individual variation in the timing of activity onset (i.e. chronotype) is increasingly studied in the wild, but we still lack a proper understanding of its adaptive significance. Using a long-term dataset we are the first to go beyond studying the relationships between chronotype and annual reproductive success, by in addition investigating its significance for survival and ultimately life time reproductive success. However, we found no significant relationships between chronotype and different measures of annual reproductive success (i.e. annual number of eggs and fledglings), both at the among- and within-individual level, between chronotype and longevity (i.e. age at death), or between chronotype and lifetime reproductive success (i.e. life time number of eggs, fledglings or recruits). Below we discuss the possible causes and consequences of our findings.

Timing of activity onset and reproductive success

Our study, that was based on a substantial, long-term dataset of individually marked great tits, did not provide any evidence that emergence time is related to annual reproductive success. This finding is, among others, different from the outcome of a recent study by Womack et al. (2023a), who found that female great tits with an earlier onset of activity in the morning raised more fledglings. This was found in both urban and rural populations, so that it is unlikely that the differences between studies are due to habitat, i.e. our population is situated in a sub-urban environment. However, selection pressures on chronotype may change over time, differ between populations and vary with environmental factors that were not considered in either of the studies. Nevertheless, our results were in agreement with studies in European starlings and blue tits (Maury et al., 2020; Steinmeyer et al., 2013). Thus, across species evidence is accumulating that individual variation in emergence time does not relate to annual reproductive success.

The long-term data set further allowed us to investigate associations between chronotype (i.e. emergence time) and longevity and ultimately life time reproductive success. This has to the best of our knowledge never been done in free-living animals, but it could be more closely linked to an individual's fitness than annual reproductive success (McCleery et al., 2004). However, we did not find any effects of the timing of activity onset on lifetime reproductive success, nor was there any effect on longevity, as could have been expected in a trade-off framework. Chronotype was thus not subjected to directional selection, at least in females. Despite the fact that we could analyse a long-term data base, it has to be taken into account that in case of the lifetime number of recruits, we used a minimum estimate, as we only counted local recruits and could not determine emigration rates (Brommer et al., 2004; McCleery et al., 2004). The results for the lifetime number of recruits should thus be interpreted with caution.

Lack of selection on emergence time

While there was no evidence for directional selection on chronotypes, we also did not find indications for stabilising or disruptive selection. Individuals with emergence times closer to the population mean were equally successful as individuals with more extreme emergence times, i.e. very early (up to 120 minutes before sunrise) or very late chronotypes (up to 90 minutes after sunrise). There is hence significant variation in emergence times in our population, without being linked to reproductive success and survival. The variation could be maintained via balancing selection (Brommer & Class, 2017), of which frequency dependent selection is an example. Here the fitness consequences of a certain trait depend on the frequency of that trait within the population (Wolf & McNamara, 2012). In the case of chronotypes, being early may only be advantageous, if the rest of the population is relatively late, so that an individual could for example forage before the others. This could lead to selection for earlier chronotypes until this advantage disappears when too many other individuals are early too. Additionally, depending on the environment in which an individual lives, selection pressures on the timing of activities may differ, resulting in spatially or temporarily fluctuating selection pressures (Smith & Blumstein, 2008). Unfortunately, we could not investigate the differences in the relationship between chronotype and reproductive success and survival between years, due to convergence problems with the models, which would have enabled us to explore this possibility by linking it to among year variation in environmental variables. However, even within populations individuals may occupy different ecological niches and trait optima may thus differ among individuals based on their individual niche specialisation (matching habitat choice; Bolnick et al., 2003; Edelaar et al., 2008), so that one may have to manipulate environmental factors at the nest level in order to fully understand the observed patterns. Finally, chronotype could be part of a larger (behavioural) syndrome, where different traits are correlated with each other on the among individual level (e.g. chronotype and aggression) as we could show in one of our earlier studies (Meijdam et al., 2024), which may constrain its evolution. Similarly, selection could differ between the sexes, as earlier timing of male dawn song is related to more extra pair copulations and hence potentially fitness, while we did not find directional selection pressures on the timing of activity onset in females. Still, such constraining processes could maintain trait variation.

Clutch initiation date

Females that started clutch initiation earlier laid more eggs and raised more fledglings. The timing of breeding could be indicative for an individual’s circannual timing (i.e. annual chronotype), which has been shown to be linked to an individual’s circadian timing (Graham et al., 2017; see also Liedvogel et al., 2009 and Liedvogel & Sheldon, 2010). However, in our population we already showed that emergence times were not related to the clutch initiation date in females (Meijdam, Müller, Thys, et al., 2022). As we included second clutches in our parameters for annual reproductive success, one could argue that the relationship between clutch initiation date and number of eggs and fledglings is mainly determined by whether or not a female initiated a second clutch, since delaying the first clutch decreases the chance of starting a second one

(Barba et al., 1995; Senécal et al., 2021). However, the relationship remained significant for the number of eggs when only first clutches were included, but not for the number of fledglings (Table S1). This relationship may reflect condition or quality differences among females or quality differences in the breeding territory, which are not mutually exclusive (Christians et al., 2001; see also Garamszegi et al., 2004; Verhulst et al., 1995).

Conclusions

Here we studied the consequences of variation in the timing of activity (here: emergence times) in free-living animals for survival and for both annual and lifetime reproductive success However, we did not find evidence on the adaptive significance of among-individual variation in chronotypes. Furthermore, we did not find any signs of stabilising, directional or disruptive selection. However, in order to (dis)prove a number of alterative hypotheses, future research should integrate the environment at the individual level and possible differences in selection pressures between the sexes, to further explore the lack of consequences for reproductive success and survival in females as observed in this study.

Supplementary material

The supplementary information accompanying this chapter can be found on pages 194 – 196 of this thesis.

Author Contribution Statement

Marjolein Meijdam: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – original draft.

Marcel Eens: Conceptualisation, Methodology, Writing – review & editing.

Wendt Müller: Conceptualisation, Methodology, Writing – review & editing.

Acknowledgements

We thank Peter Scheys and Geert Eens for their field assistance. This work was supported by the Biology Department of the University of Antwerp (Dehousse grant to Marjolein Meijdam) and FWO Flanders (FWO projects ID: G0A3615N and G052117N to Marcel Eens and Rianne Pinxten). We thank both for their support.

Chapter 7

“Early to bed, but not early to rise” is associated with increased reproductive success and survival in male great tits

Meijdam, M., Eens, M., & Müller, W.

Abstract

Individual variation in the daily timing of activity, i.e. chronotypes, may lead to among individual differences in exposure to various risks and opportunities throughout the day, such as predation risk, food availability and mating opportunities, potentially with consequences for fitness. For example, in male songbirds, earlier timing of activity onset in the morning has been related to increased numbers of extra pair offspring. However, relationships between other fitness components, such as reproductive success and survival have never been explored. Here, we investigate the associations between the timing of activity onset, the timing of activity offset, the active daylength and reproductive success and survival in male great tits. To determine these timing parameters, we measured the emergence time from the nest box in the morning and entry time into the nest box in the evening in January. Emergence time was not related to reproductive success and survival , but males with earlier entry times into the nest box had partners with earlier laying dates, higher probabilities of starting a second clutch, more fledglings (when replacement clutches and second clutches are included) as well as higher survival probability. Active daylength was strongly correlated with entry time, so we found similar, yet less significant results. Furthermore, the timing of activity onset was not correlated to the timing of activity offset on the among individual level. Our study is the first to show such strong correlations between the timing of activity offset and reproductive success and survival in males, while in females this association is absent. Hence, sex differences in the optimal time window for activity may exist.

Introduction

Individual differences in daily timing of behaviour, i.e. so-called chronotypes, have been observed in many species (e.g. Alós et al., 2017; Chmura et al., 2020; Meijdam et al., 2022; Refinetti et al., 2019; Schlicht & Kempenaers, 2020). In humans, for example, individuals with early chronotypes wake up earlier and go to bed earlier than individuals with late chronotypes (e.g. Korczak et al., 2008; Roepke & Duffy, 2010). In free-living animals, chronotypes are typically determined by measuring the timing of activity onset in the morning and/or activity offset in the night (Dominoni et al., 2013; Maury et al., 2020; Meijdam, Müller, Thys, et al., 2022; Strauß et al., 2022; Womack et al., 2023a). Timing can be mechanistically underpinned by the circadian clock (Cassone, 2014; Helm et al., 2017) and may have functional consequences, as risks and opportunities vary throughout the day. For example, in many bird species, copulations occur early in the morning, and waking up late may thus lead to lower reproductive output, or to higher cuckoldry risks (Hau et al., 2017). On the other hand, predation risks may be highest during dawn and dusk, when many predators are active and vision is still impaired. Waking up early may thus lead to increased mortality (Helm & Womack, 2018). Hence, it is likely that variation in the timing of activity onset and offset has consequences for fitness.

So far, studies on the adaptive significance of variation in timing of activity have mainly focussed on extra pair paternity in birds. Males that initiated the dawn song or activity earlier in the morning had higher probabilities of gaining extra pair young and lower risk of being cuckolded (Dolan et al., 2007; Greives et al., 2015; Poesel et al., 2006; Santema & Kempenaers, 2023; Schlicht et al., 2023; Steinmeyer et al., 2013). Higher extra pair paternity rates due to early diel timing may lead to higher reproductive output in males, but this has never been investigated. Yet, for females chronotype was not related to extra pair paternity (Schlicht et al., 2014; Steinmeyer et al., 2013) and it is also not related to reproductive success or survival (Maury et al., 2020; Steinmeyer et al., 2013; but see Womack et al., 2023a). However, as the timing of activity onset and offset often differs between males and females (Schlicht & Kempenaers, 2020; Stuber, Dingemanse, et al., 2015; Stuber, Mathot, et al., 2015), it is possible that the trait optima for timing of activity differ among the sexes. Consequently, the direction and magnitude of selection may also be different, but investigating this requires additional data on reproductive output, other than extra pair young, and survival in relation to chronotype in males.

Therefore, we here investigate the effects of the timing of activity onset, the timing of activity offset and the active daylength on the reproductive success and survival of male great tits. To determine the timing of activity we measured the emergence time from the nest box in the morning and the entry time into the nest box in the evening, right before the start of the breeding season. During the breeding season we collected data on the laying date of the first egg, the number of eggs and fledglings in the first clutch, whether there was a second clutch, the total number of eggs and fledglings (including replacement and second clutches) and whether individuals survived the period between the timing measurements in winter and the breeding season. Furthermore, we also investigated whether the timing of activity onset is related to the timing of activity offset, both at the among and within individual level.

Methods

This study was carried out in a suburban, nest box breeding great tit population in Antwerp, Belgium (51°09’46.1"N, 4°24’13.3"E), that has been monitored since 1995 (Dauwe et al., 1999). Each year, individuals were captured when roosting in the nest box in winter, during which their age (yearling or older) and sex were determined based on plumage characteristics. To facilitate individual recognition, individuals received a metal ring, two colour rings and a PIT-tag (passive integrated transponder) around their tarsi. Individuals not captured during winter were caught on the nest during the chick rearing phase of the breeding season, when the chicks were at least 8 days old. All nestlings were ringed at day 14 or 15, so for many recruits in the population the age is known. We routinely determined clutch initiation dates, clutch sizes and the number of fledglings. In our population replacement clutches as well as second clutches occur and clutch sizes and number of fledglings were determined as for the first clutch.

Ethical note

This study was approved by the ethical committee of the University of Antwerp (ID numbers: 2016-87, 2017-23, 2017-61 and 2018-50) and was performed in accordance with Belgian and Flemish laws regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complies with ARRIVE guidelines. The Royal Belgian Institute of Natural Sciences (KBIN) provided ringing licenses for all authors and technicians. Handling time was minimized as much as possible. All other methods described above are non-invasive.

Emergence time

In January 2023, all nest boxes of the population were checked during the night (between 06:00 and 10:00 p.m.) for the presence of male great tits. A handheld transponder reader (GR-250 RFID Reader, Trovan, Aalten, Netherlands) was used, which registered unique PIT-tags, enabling individual recognition without disturbance. The day after, SongMeters were placed on top of nest boxes in which males were roosting during the night to determine male activity onset, activity offset and active daylength, by measuring the emergence time from the nest box in the morning and the entry time in the evening ($N_{emergence}$ = 313, N_{entry} = 319 on 52 males; SongMeterTM SM2+; Wildlife Acoustics, Inc, U.S.). All measurements were taken between January 17 and January 31. One microphone of the SongMeter was placed inside the nest box and the other microphone outside. Recording sessions were conducted from 06:30 to 09:00 a.m. CET and from 03:30 to 06:30 pm. When an individual leaves or enters the nest box the sound of their wings, their claws on the nest box (Halfwerk et al., 2011) and the male passing through the opening of the nest box can be heard. Emergence and entry time were determined using Avisoft SASLab Pro 5.2.14 (Specht, 2002), and active daylength was calculated based on the emergence and entry times. We determined emergence and entry time relative to sunrise and sunset (negative = before sunrise/sunset, positive = after sunrise/sunset) and active daylength relative to the daylight period (negative = shorter active than the daylight period, positive = longer active than the daylight period), and hereafter, emergence time, entry time and active daylength concern relative times. Temperature data was retrieved via:

https://www.wunderground.com/history/daily/be/antwerp. In total, we measured each male on average on 6.02 ± 0.70 days for emergence time, on 6.02 ± 1.05 days for entry time and on 4.88 ± 0.65 for active daylength.

Statistical analyses

All statistical analyses were performed in R 4.1.3 (R Core Team, 2013). The MCMCglmm package (version 2.35) was used throughout to fit models (Hadfield, 2010). First, we constructed a bivariate mixed effect model to study the covariation between emergence time and entry time (Houslay & Wilson, 2017). We included a polynomial date effect up to the second order and age (yearling vs older) for both dependent variables. The temperature at sunrise was included at the level of emergence time and the temperature at sunset for entry time (Meijdam et al., 2024; Meijdam et al., 2022; Schlicht & Kempenaers, 2020). Male identity was included as random effect, which enabled partitioning the (co)variance into the among- and within-individual components (Dingemanse & Dochtermann, 2013). The among- and within-individual covariances were converted into correlations by dividing the respective covariance by the square root of the product of the respective variances. Including active daylength in this model as dependent variable led to convergence problems. Therefore, a second and a third bivariate mixed model were constructed to study the covariance between emergence time and active daylength and between entry time and active daylength. These models included the same fixed and random effects as described above. The maximum daily temperature was included as fixed effect at the level of active daylength. For emergence and entry time, repeatability was calculated based on the model that included both. Repeatability of active daylength was calculated based on the model that included emergence time and active daylength.

To study the correlation between respectively emergence time, entry time or active daylength and reproductive success and survival, we mean centred all three variables within days (each day data for at least 21 males were available for all three variables).

For each individual we used the average of the mean centred values. To determine reproductive success we used the clutch size of the first clutch, the number of fledglings of the first clutch and the total number of eggs and fledglings produced (i.e. sum of the first clutch, replacement clutch and second clutch). We also determined the laying date of the first egg of the focal male's partner and whether or not a second clutch was initiated (i.e. after a successful first clutch, so replacement clutches were not included) as these are important reproductive decisions. Furthermore, we determined survival (i.e. a male was considered dead if it was not found breeding in the population and was also not encountered during night checks in the next winter). For each of these variables we constructed three univariate models, one with the average emergence time as fixed effect, one with the average entry time as fixed effect and one with the average active daylength as fixed effect. These models also included age (yearling vs older) as fixed factor. For the laying date we used a gaussian distribution, for clutch size, the number of fledglings and the total number of eggs and fledglings we used Poisson distributions and, as binomial distributions are not available in MCMCglmm, we used a categorical distribution together with a prior that fixed the residual variance at 1 for the presence of second clutches and survival (see supplementary material for prior specifications).

All multivariate models were run with Gaussian error distributions. We set the number of iterations at 850 000, the burn-in phase at 50 000 and the thinning interval at 400. The use of alternative prior specifications (i.e. Inverse Wishart and Inverse Gamma) gave qualitatively similar results. Traces of posterior distributions were checked visually and autocorrelation between successively stored iterations was less than 0.1 in all cases (Hadfield, 2010). Model convergence and mixing was assessed using Gelman Rubin statistics between chains (i.e. the potential scale reduction factor was <1.1 in all cases; Gelman & Rubin, 1992). Results presented are posterior mean estimates with associated 95% credible intervals (CrI). Fixed-effects and correlation estimates were considered to find strong support if 95% CrI did not overlap with zero.

Results

Emergence times ranged from 87.6 minutes before sunrise up to 3.1 minutes before sunrise (mean ± SD = -50.3 ± 11.5). Entry times ranged from 49.3 minutes before sunset up to 24.7 minutes after sunset (mean ± SD = -2.4 ± 13.9) and daylengths ranged from 6.2 minutes shorter than the daylight period up to 86.3 minutes longer (mean ± SD = 47.6 ± 18.1). All three variables were affected by date in a non-linear way (Table 1). Emergence times became earlier on later dates, while entry times became later and, consequently, active daylength lengthened. Temperature only affected entry times, with males entering the nest box later on warmer nights. Age did not affect emergence time, entry time or active daylength. All three timing of activity variables were repeatable ($R_{\text{emergence time}}$ = 0.77 [0.69, 0.85], $R_{\text{entry time}}$ = 0.38 [0.25, 0.51], $R_{\text{active daylength}}$ = 0.38 [0.26, 0.51]). Emergence and entry time were not correlated at the among individual level (0.23 [-0.08, 0.54]), but at the within individual level they were negatively correlated (-0.32 [-0.43, -0.20]). Emergence time and active daylength were negatively correlated at both the among (-0.66 [-0.86, -0.44]) and within individual level (-0.68 [-0.74, -0.60]) and entry time and active daylength were positively correlated at both levels (among: 0.53 [0.29, 0.76], within: 0.91 [0.89, 0.94]).

The laying date of the first egg was positively correlated with the male's entry time into the nest box and active daylength, but not with emergence time (Table 2, Figure 1). Males with earlier entry times and shorter active daylengths had partners that initiated egg laying earlier in the season than males with later entry times. Clutch size and number of fledglings of the first clutch were independent of emergence time, entry time and active daylength. Furthermore, males with earlier entry times and shorter active daylength had higher probabilities of starting a second clutch. The total number of eggs was not related to emergence time, entry time and active daylength, but the total number of fledglings was larger for males with earlier entry times. Finally, males with earlier entry times had higher survival rates than males with later entry times

(Table 2, Figure 1). The difference in entry times between males that survived and males that did not survive was on average 11.3 minutes.

Table 1: Results from bivariate mixed models with emergence time from the nest box (in minutes relative to sunrise), entry time into the nest box (in minutes relative to sunset) and active daylength (in minutes relative to the daylight period) as dependent variables. Estimates of fixed (β) and random (σ^2) components are shown with 95% credibility intervals. Fixed effects that found strong support are presented in bold.

	Emergence time[1]			Entry time[2]			Daylength[3]		
Fixed effects	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl
Intercept	**-50.09**	**-56.04**	**-44.81**	-1.98	-6.79	3.22	**50.26**	**41.88**	**57.14**
Date	-25.49	-68.06	11.08	-4.53	-63.40	52.79	31.86	-49.68	113.2
Date 2	**-87.40**	**-108.75**	**-63.09**	**124.60**	**84.30**	**162.54**	**212.1**	**149.7**	**273.7**
Age	-0.70	-7.55	5.57	-3.05	-8.73	2.45	-2.50	-9.68	5.32
T sunrise	0.13	-0.27	0.55	-	-	-	-	-	-
T sunset	-	-	-	**0.88**	**0.28**	**1.40**	-	-	-
T max	-	-	-	-	-	-	0.18	-0.70	1.11
Random effects	*σ^2*	Lo. Crl	Up. Crl	*σ^2*	Lo. Crl	Up. Crl	*σ^2*	Lo. Crl	Up. Crl
MaleID	114.49	68.60	163.37	69.06	36.04	105.61	123.3	63.71	188.21
Residual	32.88	27.29	38.80	108.72	89.70	126.63	196.85	159.42	234.04
Covariance among individuals	20.13[a]	-10.23	49.96	49.40[b]	13.12	87.71	-78.2[c]	-125.71	-35.21
Covariance within individual	-19.16[a]	-26.84	-10.65	130.3[b]	107.39	155.4	-54.68[c]	-67.11	-42.15

[1] results from the model with emergence and entry time

[2] results from the model with emergence and entry time

[3] results from the model with emergence time and daylength

[a] covariance between emergence and entry time

[b] covariance between entry time and daylength

[c] covariance between emergence time and daylength

Table 2: Results from (general) linear models testing the correlation between respectively male emergence time, entry time or active daylength and reproductive success and survival. Estimates of fixed (β) and random (σ²) components are shown with 95% credibility intervals and fixed effects that found strong support are presented in bold. In Second clutches only ‘true’ second clutches are included (i.e. first brood was successful, no replacement clutches included). For the Total number of eggs and Total number of fledglings both replacement clutches and second clutches were included.

	Laying date			Clutch size (first clutch)			Fledglings (first clutch)			Second clutch		
Fixed effects	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl
Emergence time	-0.36	-0.93	0.13	0.00	-0.02	0.02	0.00	-0.04	0.02	0.18	-0.02	0.40
Age	-3.23	-10.16	4.09	-0.05	-0.31	0.24	-0.23	-0.59	0.12	-0.14	-2.34	2.24
Entry time	**0.44**	**0.09**	**0.80**	0.00	-0.02	0.01	-0.02	-0.04	0.002	**-0.17**	**-0.33**	**-0.04**
Age	-3.48	-9.54	4.00	-0.05	-0.30	0.24	-0.23	-0.57	0.11	-0.03	-2.51	2.27
Daylength	**0.32**	**0.05**	**0.62**	0.00	-0.01	0.01	-0.01	-0.02	0.01	**-0.16**	**-0.28**	**-0.03**
Age	-3.76	-10.17	3.50	-0.05	-0.31	0.23	-0.22	-0.55	0.12	0.06	-2.50	2.29

	Total number of eggs			Total number of fledglings			Survival		
Fixed effects	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl	*β*	Lo. Crl	Up. Crl
Emergence time	0.01	-0.01	0.03	0.00	-0.02	0.02	-0.04	-0.14	0.05
Age	0.14	-0.14	0.43	-0.05	-0.33	0.26	1.46	-0.25	2.98
Entry time	-0.01	-0.03	0.0005	**-0.02**	**-0.04**	**-0.01**	**-0.31**	**-0.50**	**-0.12**
Age	0.14	-0.12	0.41	-0.05	-0.34	0.25	0.95	-1.09	3.23
Daylength	-0.01	-0.02	0.0007	-0.01	-0.02	0.0004	-0.07	-0.15	0.004
Age	0.15	-0.14	0.41	-0.04	-0.33	0.25	1.54	-0.02	3.34

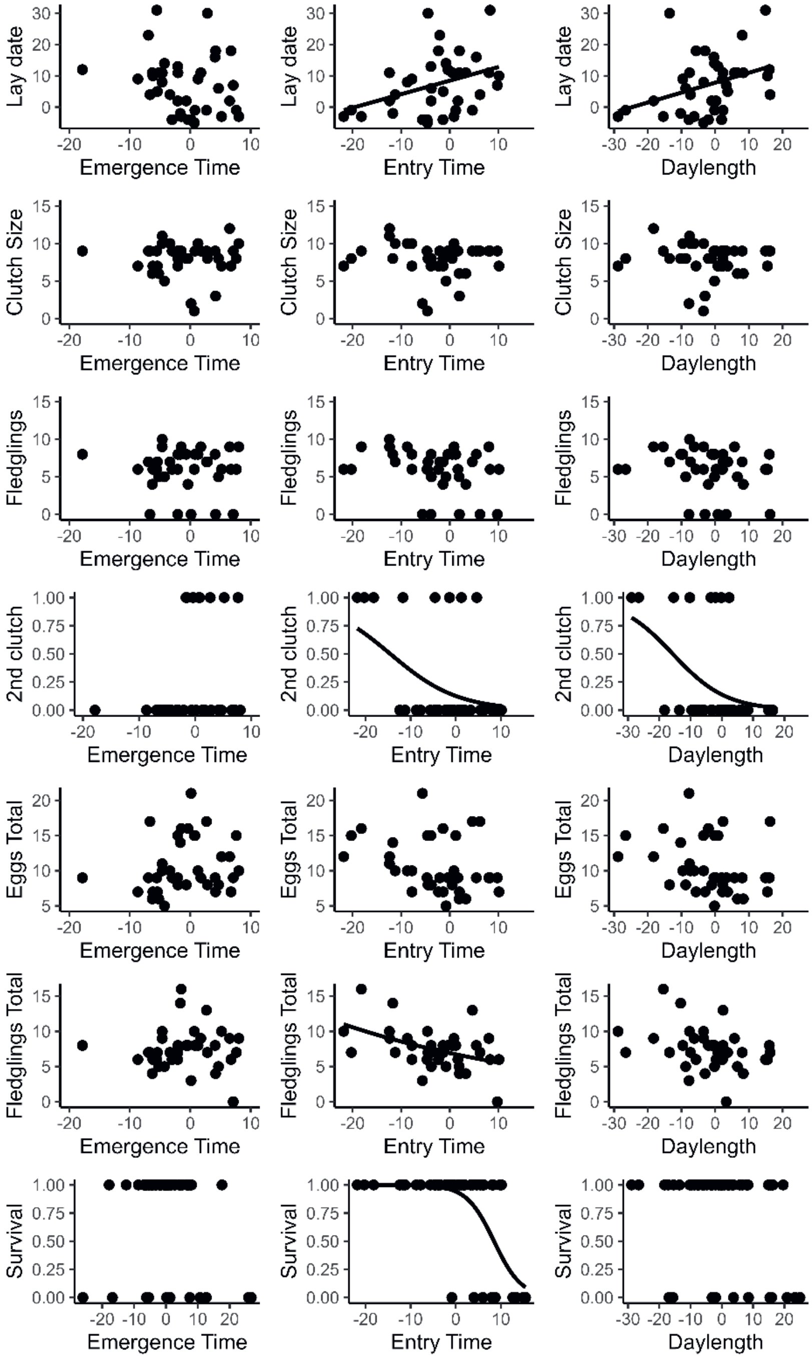


Figure 1: The correlations between respectively male emergence time, entry time or active daylength (mean-centred within days and averaged per individual) and reproductive success and survival.

Discussion

We tested whether individual differences in the timing of activity were associated with differences in the reproductive success and survival of male great tits. Males with later entry times had partners that initiated egg laying later in the season, they were less likely to initiate a second clutch and they had in total fewer fledglings than males with earlier entry times. They were also less likely to survive the period between the timing measurements in January and the breeding season. Surprisingly, emergence time did not have any effect on reproductive success or survival.

Consequences of the timing of activity for reproductive success and survival

Even though the daylight period is relatively short in January, we found significant variation among individuals in all three timing variables. The among individual variation in entry time and active daylength was associated with multiple fitness components. Yet, as entry time was correlated with active daylength and all relationships were stronger for entry time, we assume that it is mainly the entry time in the evening that determined the reproductive success and survival. One possible explanation for the reported relationships is that there may be directional selection for earlier entry times in our population. Because great tits have impaired vision during dawn and dusk (Kacelnik, 1979), they may experience greater threats from predators, and hence avoid twilight conditions. Only birds in poorer condition or those breeding in poor quality habitats may be forced to feed during this period, if they cannot obtain enough resources during the day light period. Indeed, Krams (2000) showed that dominant individuals had shorter feeding day lengths than subordinates, starting foraging later and ceasing earlier, when night time predators (here pygmy owls, *Glaucidium passerinum*, which especially hunt during twilight) were present. In our population, tawny owls (*Strix aluco*) and domestic cats (*Felis silvestris catus*), are common nocturnal predators, and males that entered the nest box later may thus have had a higher probability of being predated upon, which could have resulted in lower survival (Curio et al., 1983; Pavisse et al., 2019; Sasvári & Hegyi, 1998). These males may have been subordinates, or birds in poor condition, which could have forced them to forage at risky hours and their hierarchical position or condition could have affected their reproductive output too. However, why we did not find a similar effect on emergence times remains unclear.

Laying date, clutch size and reproductive success

The associations between entry time and reproductive output that we observed, may all be related to the laying date of the first egg. In fact, initiating the first clutch earlier increases the likelihood of starting a second clutch, because for early laying couples the food availability may still be high enough to start a second clutch after the first nest is fledged, while for late breeders the food availability is too low (Barba et al., 1995; Senécal et al., 2021). Starting a second clutch may in turn increase the total number of fledglings. When interpreting these findings one has to take into account that this may be an indirect relationship, because the laying date is most likely strongly determined by the female (but see Evans et al., 2020), of which we do not have chronotype measures. Interestingly, in blue tits (*Cyanistes caeruleus*), assortative mating for awakening times was found and a similar trend for sleep onset was present (Steinmeyer et al., 2013), which possibly explains the correlation between male entry time and a female trait (i.e. laying date). Another potential mechanism by which males can affect the laying date of the female is stimulation through singing and courtship feeding (Kroodsma, 1976; Verhulst, 2003). As we argued above, early entering males may be in better condition or of higher quality, and interestingly singing performance in males may be related to the males quality or condition too (Rivera-Gutierrez et al., 2010). The males quality/condition may thus indirectly affect the laying date. Correlations between timing of activity and respectively laying date and the initiation of a second clutch have been observed before in females but for onset of activity, not offset. In dark-eyed juncos (*Junco hyemalis aikeni*) and great tits, females that started activity earlier during the day initiated egg laying earlier (Graham et al., 2017, but see Meijdam, Müller, Thys, et al., 2022) and female European starlings (*Sturnus vulgaris*) that initiated a second clutch were active earlier during the day than individuals that did not start a second clutch.

Individual differences in the timing of activity in male songbirds have been related to increased extra pair offspring, but whether this affected their reproductive success is unknown. Here, we unfortunately do not have information on extra pair paternity. However, there may be a trade-off between investment in the nest and investment in gaining extra pair paternity (i.e. parenting-mating trade-off; Lv et al., 2020; Magrath & Komdeur, 2003; Stiver & Alonzo, 2009). Potentially, males with later entry times compensated their low reproductive success by siring more extra pair offspring. Yet, in blue tits, late activity offset was related to a lower likelihood of gaining extra pair paternity (Schlicht et al., 2023; Steinmeyer et al., 2013), making this scenario highly unlikely.

Trait correlations

Although in humans individuals with early chronotypes wake up and go to bed earlier, we did not find such a correlation between the timing of activity onset and offset in male great tits (see also Maury et al., 2020 and Schlicht et al., 2023 for similar results in European starlings and blue tits). Potentially, the timing of activity onset and offset are not only affected by the circadian clock, but also by differences among individuals in sensitivity to light (Womack et al., 2023a) and in energetic requirements. Furthermore, in mammals sleep homeostasis affects the circadian rhythm too, by increasing the sleep pressure throughout the day, depending on the amount of time spent awake (Huang et al., 2011; Jagannath et al., 2021). The timing of activity onset and offset may thus be the result of a complex interplay between these modulating processes, leading not only to differences in chronotype, but also to differences among individuals in the active daylength. At the within individual level we found a negative correlation between the timing of activity onset and offset, which confirmed the results from Stuber et al. (2015). This negative correlation may arise because of variation in weather conditions between days (Rao, 2021; Schlicht & Kempenaers, 2020). For example, on rainy days, the timing of activity onset is delayed and the timing of activity offset advanced.

Conclusions

Overall, we found negative associations between later activity offset and male reproductive success and survival in great tits. Later entering males had clutches with later laying dates, fewer second clutches, and less fledglings, as well as lower survival to the next breeding season. As the underlying mechanisms that determine entry times are not yet fully understood we can only speculate that the variation could be predominantly related to individual condition, and hence the time needed to accumulate sufficient resources. Our results are surprising since it has been suggested that mainly the morning hours are ecologically and evolutionarily relevant for songbirds, as territory defence, mate attraction and copulation mainly occur in the morning and the need to replenish energy stores during this part of the day is high (Dominoni et al., 2013; Lehmann et al., 2012; Womack et al., 2023a). Here, we actually show the contrary. When additionally considering our finding that the timing of activity onset is not related to the timing of activity offset at the among individual level, we think it is essential for future research to not only consider the timing of activity onset when studying chronotypes, but also the timing of activity offset and active day length. In females, studies considering the timing of activity offset in relation to fitness are scarce, but so far, no significant associations have been reported (Steinmeyer et al., 2013). This may indicate that the selective forces that shape the timing landscape may differ between the sexes, which highlights the need for a sex-specific approach.

Supplementary material

The supplementary information accompanying this chapter can be found on page 197 of this thesis.

Author Contribution Statement

Marjolein Meijdam: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – original draft.

Marcel Eens: Conceptualisation, Methodology, Writing – review & editing.

Wendt Müller: Conceptualisation, Methodology, Writing – review & editing.

Acknowledgements

We thank Peter Scheys and Geert Eens for their field assistance. This work was supported by the Biology Department of the University of Antwerp (Dehousse grant to Marjolein Meijdam) and FWO Flanders (FWO projects ID: G0A3615N and G052117N to Marcel Eens and Rianne Pinxten). We thank both for their support.

Chapter 8: General discussion

Differences between individuals in the timing of their behaviour are ubiquitous, but it is as yet unclear how such individual differences arise and, even more so, what their functional consequences are. At the same time, due to human activity, major changes are taking place in animal habitats that may affect the daily timing of activities through interference with the circadian clock, e.g. due to light pollution, which may lead to mistimed behaviours. To complicate matters further, the (daily) timing of activities might also be intertwined with the annual timing of reproduction, which again may be affected by anthropogenic effects, such as the need for earlier timing of reproduction due to climate change. These examples illustrate the need for better knowledge of the adaptive significance of timing in the wild, to understand the potential risks and to predict the fate of populations or species.

In this thesis, I therefore studied whether individual great tits are consistent in their daily timing of activity and how their timing varies on temporal and spatial scales. In addition, I examined what consequences individual differences in the daily timing of activity have on the reproductive success and survival of great tits, both in females and in males. Fitness consequences could potentially arise from the daily opportunities and risks that have to be weighed against each other, but there is also evidence that the circadian clock, as mentioned above, affects the annual timing of reproduction, which in turn could determine fitness, as the optimal timing of reproduction is generally related to higher reproductive success (e.g. Garamszegi et al., 2004; Verhulst et al., 1995). Moreover, different (behavioural) traits are often not expressed independently of each other and a given combination of different traits may be important for fitness than any trait on its own. Therefore, I studied the correlation between the daily timing of activity and the annual timing of reproduction, as well as the correlation between the timing of activity and aggression. Finally, I also studied possible effects of anthropogenic disruption of sleep-wake rhythms, in this case exposure to artificial light at night, on cognitive performance, as this may define the impact of anthropogenic effects.

Individual variation in the timing of activity

Variation between individuals in phenotypic traits is the raw material on which selection can act (Darwin, 1859; Endler, 1986; Lynch et al., 1998), yet to actually evolve, this variation must be heritable. For chronotypes in birds, as for most behavioural traits, we do not know exactly how individual differences arise and which genes are responsible for them and to what extent. Therefore, in practice, repeatability estimates (i.e. the amount of variation in a population that is explained by differences between individuals relative to the total variation) are often used to determine whether variation in a trait has an intrinsic underlying component (Dingemanse & Dochtermann, 2013). In this thesis, repeatability for timing of activity onset was calculated several times and over different time periods. In **Chapter 2**, the repeatability of emergence time during the pre-egg laying period in females was 0.39 [0.10, 0.62], in **Chapter 7** it was 0.77 [0.69, 0.85] in males in winter, and in **Chapter 3**, it was found to be 0.46 [0.39, 0.59] in females within the egg laying period (all repeatability data are in the short term i.e. within years). Additionally, repeatability of emergence times in females during the egg laying period was significant between years (R = 0.27 [0.16, 0.34]). As discussed in **Chapter 3**, this indicates the presence of intrinsic, likely genetic, differences between great tits that determine the timing of activity onset, although persistent early environmental effects and maternal effects may play a role too. However, the fact that the long-term repeatability seems to be lower than the short-term repeatability indicates that environmental effects play a role too, and that their changes on a temporal scale influence the timing of activity onset. Temporal autocorrelation between emergence times measured close in time may also stem from the state of an individual that can affect its emergence time, as states are likely relatively stable over shorter time scales. For example, immune challenges, nutritional condition and hormonal state may affect the diurnal pattern of activity (Elderbrock et al., 2021; Graham et al., 2019; Lennon et al., 2023, see also **Box 1**).

Temporal changes were also observed within breeding seasons in **Chapter 2** and **Chapter 3**, when all females were measured during the start of the egg laying period. In both cases, the timing of activity onset became later on later dates, and in **Chapter 2** this was accompanied by earlier entry times and longer active daylengths. The change in all three parameters at roughly the same time (i.e. around the 10th of April) could likely be due to a change in the environment (e.g. food availability), rather than to intrinsic differences in the timing of activity onset between early and late laying individuals, which supposedly would give a more gradual change over time. Thus, it seems that temporal changes in the environment can be very important in determining the timing of activity onset (Schlicht & Kempenaers, 2020; Stuber, Dingemanse, et al., 2015; Womack et al., 2023a). Intriguingly, spatial variation appeared to play a lesser role, since the nest box location (i.e. spatial variation) did not explain any of the variation in the timing of activity onset, which was investigated via repeated measurements of individual females that were each measured in more than one nest box as well as multiple females that were measured in the same nest boxes (**Chapter 3**). These findings are extremely important when interpreting the effects of timing of activity on fitness, because when individuals are measured at different moments in time, their timing of activity may reflect the environmental conditions rather than an individual's chronotype, which could lead to confounded conclusions (**Chapter 2**).

The impact of season, i.e. temporal scales, on the timing of activity is further emphasized by the fact that the timing of activity onset in females in **Chapter 2** was not repeatable between different periods of the year (i.e. pre-egg laying and egg laying period), although admittedly, this result is based on a small sample size. However, a study in European starlings has also examined repeatability between different reproductive periods (i.e. incubation and chick-rearing) and there too it was not significant (Maury et al., 2020). Between different (reproductive) periods individuals seem to change their timing of activity, potentially to match the demands of a certain period (see also **Box 1**; Schlicht & Kempenaers, 2020; Stuber et al., 2015), but most importantly individuals appear to differ in their responsiveness to such changing demands. To increase our understanding of the adaptive significance of the timing of activity, follow-up research could investigate which environmental factors (e.g. predation risk or social density) and demands (e.g. food provisioning for chicks) affect the timing of activity and how individuals differ in their plastic responses, adopting a behavioural reaction norm approach (see also Dingemanse et al., 2010).

Not only the timing of activity onset, but also the timing of activity offset may be affected by environmental variation, yet differently. Anticipation for example, which is considered as one of the most important functions of the circadian clock (Deans, 2021; Green et al., 2002), might be more important for the correct timing of activity onset than for the timing of activity offset. This is particularly true for cavity nesting and burrowing species, as when roosting inside cavities and burrows in the morning, fluctuating environmental variables can be observed to a lesser extent (Everts et al., 2004; Hut et al., 1999). In cavity nesting and burrowing species, it is therefore likely that emergence time more strongly reflects an intrinsic component than entry time, where it is overlayered by environmental effects (Chmura et al., 2020; Lehmann et al., 2012). There may also be differences between the demands and potential risks in the morning and the evening. In songbirds, territory defence, mate attraction and copulation mainly occur in the morning and the need to replenish energy stores during this part of the day is high. Furthermore, perceived predation risk may be higher when emerging from the nest box compared to when entering, as the possibilities of predator detection from the nest box are limited. Correct timing may thus be more ecologically and evolutionarily important in the morning (Dominoni et al., 2013; Lehmann et al., 2012; Womack et al., 2023a). Accordingly, previous studies have shown that the timing of activity offset in the evening is more sensitive to weather conditions, such as temperature, than the timing of activity onset in the morning (Chmura et al., 2020;

Everts et al., 2004; Lehmann et al., 2012; Stuber, Dingemanse, et al., 2015) and that the variation in the timing of activity offset can be much larger throughout the year than the timing of activity onset (Schlicht & Kempenaers, 2020). These findings were partially confirmed in this thesis: no effect of morning temperature on emergence time was found (**Chapters 2, 3, 5, 7**), except in one case (**Chapter 6**). However, an effect of evening temperature on entry time was present for males in winter (**Chapter 7**), and for females during the pre-egg laying period (**Supplementary Chapter 2**). This relationship was absent for females during the egg-laying period (**Chapter 2**), which might be due to the generally higher temperatures during this period, that potentially never forced them to cease activity earlier, whereas in winter or early spring it may be too energetically costly to remain active later in cold temperatures. Although the timing of activity offset may be more variable than the timing of activity onset, it could still be genetically underpinned and may thus have consequences for fitness (see **Chapter 6** and **Chapter 7**).

Reproductive success and survival

Having shown that there is significant individual variation in the timing of activity in both males and females the question arises how this variation is maintained and what the consequences for fitness are. Timing of activity could be related to survival probability and reproductive success, as there might be an optimal time frame for activity, in which as many opportunities (e.g. food availability, mating opportunities) as possible are exploited, while risks (e.g. predation, cold) are minimised (van der Veen et al., 2017). However, in **Chapter 6**, such an optimal time frame for the timing of activity onset was not found for female great tits. Emergence time from the nest box in the morning was not associated with reproductive success or longevity, and individuals with more extreme chronotypes (i.e. both early and late) were equally successful and had similar survival rates to individuals with intermediate chronotypes. Hence, there was no evidence of directional or stabilising selection. Also for males directional selection on the timing of activity onset was absent, since emergence time from the nest box was not related to reproductive success or survival (**Chapter 7**). Surprisingly there was a relationship between the timing of activity offset and reproductive success and survival in males, with males with earlier entry times having higher reproductive success and higher survival rates than late males. Possibly, males with later entry times were in worse condition/of lower quality, which might have decreased their access to food throughout the day. To (partially) compensate, they could have stayed active for longer, even though this may have exposed them to increased predation risks, which in turn may have affected their survival. Staying active longer may also come with other costs such as increased energy expenditure due to colder temperatures and sleep loss, which could have negative effects on their reproductive success and survival (see also **Chapter 4** for negative effects of sleep loss). For females, no entry time data was available so unfortunately we cannot compare between males and females. Also most previous studies only investigated the effects of the timing of activity onset and not offset on reproductive success, except for Steinmeyer et al. (2013) who reported that in female blue tits the timing of sleep onset (highly correlated to entry time, r = 0.96) was not associated with laying date and the number of eggs.

As previously discussed, systematic differences exist between the timing of activity in males and females in birds, with males generally starting activity earlier and finishing later (Schlicht & Kempenaers, 2020; Stuber, Dingemanse, et al., 2015; Stuber, Mathot, et al., 2015). Interestingly, not only their behaviour is different, also underlying physiological processes may differ. For example, male great tits with the highest basal metabolic rate sleep the shortest, while females with the highest basal metabolic rate sleep the longest (Stuber, Mathot, et al., 2015), which may relate to sex differences in energy or time budgets. Such differences in the optimal time frame for activity and associated physiological processes may be the result of other sex-specific selection pressures, which may even change seasonally, such as those for higher territoriality in males and a greater contribution to parental care in females. For example, in Arctic ground squirrels, males were active until later in the day during the mating season, while females remained active longer during the lactation period (Chmura et al., 2020) and in pectoral sandpipers males that slept the least during the fertile period had the highest reproductive success, with some males spending more than 95 percent of the time awake, while females slept significantly more (Lesku et al., 2012). Thus, it is likely that the optimal timing and duration of activities is sex-specific and fluctuates over time, meaning that there is no single optimum for the timing of activity, so that contrasting processes such as sexually antagonistic selection may contribute to the maintenance of variation in the timing of activity.

Moreover, not only do sex-specific selection pressures fluctuate over time, but environmental variables, such as food availability and predation risk, also fluctuate, and consequently, optimal timing may fluctuate over time. Selection on the timing of activity may thus change in magnitude and direction, potentially leading to balancing selection (Brommer & Class, 2017; van der Veen et al., 2017). If selection changes in direction between years, it may cancel out in the long run. Thus, the lack of an overall selection pressure in a long-term dataset as in **Chapter 6** is perhaps not surprising. Unfortunately, in our analyses we could not differentiate between years, due to convergence issues. So far, only one other longer term study exists (i.e. 3 years of data), which reported a negative association between emergence time and the annual number of fledglings in females, but here too year to year differences in the direction and magnitude of selection were not investigated (Womack et al., 2023a). However, when visually inspecting the data it seems evident that the negative correlation is present only in one year, while in the other two years the correlations seem slightly positive/neutral (Womack et al., 2023b). This suggests that selection pressures may indeed fluctuate in time. A visual inspection of our own data did not reveal such year to year variation (Figure 1). In males we found a negative association between entry time and reproductive success and survival (**Chapter 7**), but whether this association is stable over time still needs to be shown. In future research, it would be pertinent to explore at least if the relationship between timing of activity and reproductive success and survival varies over time (i.e. between years), and further with which fluctuating environmental variables (e.g. predation risk, food availability and social density) it would be associated. Such investigation could provide valuable insights into the mechanisms (e.g. balancing selection and frequency dependent selection) underlying the maintenance of variation in temporal activity patterns (see Dingemanse et al., 2004).

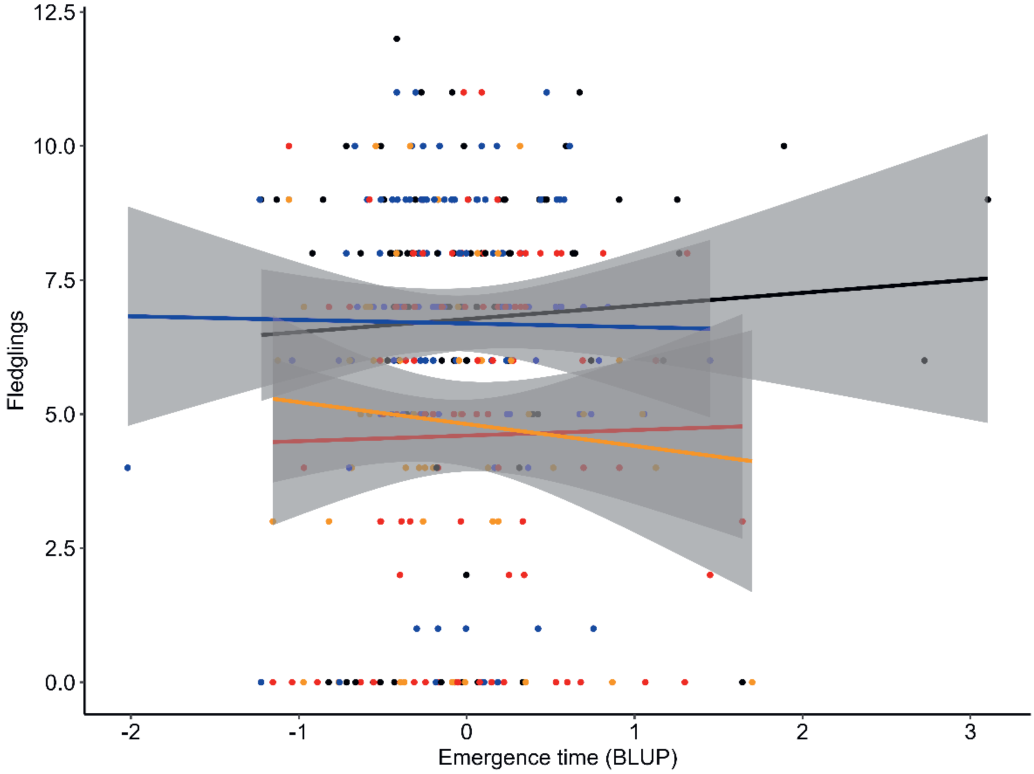


Figure 1: The correlation between female emergence time (Best Linear Unbiased Prediction based on a linear mixed effect model that included a polynomial date effect up to the second order, year, the temperature at sunrise mean centred within years and the age) and the number of fledglings in the first clutch in a given year. Black = 2018, blue = 2019, red = 2020 and orange = 2021. The data shown in this figure are part of ***Chapter 6****.*

The relationship found between the timing of activity offset and reproductive success and survival in male great tits provides insight into the effect that anthropogenic disturbance might have. As shown in de Jong et al. (2017) and Dougherty et al. (2024), anthropogenic disturbance by artificial light at night may lead to later timing of activity offset. This could be problematic, given the negative association between later entry times, reproductive success and survival found here. As discussed in **Chapter 7**, this negative association could be related to the condition/quality of males, but it is still unclear what exactly is cause and effect in this association. Males in poor condition could be forced to stay active until later in the day and their poor condition could simultaneously affect their fitness, but it is also possible that being active late in the day directly affects fitness, for example through increased exposure to predation. Including information on the condition of the individuals in the analyses, such as their body mass or physiological condition or experimental manipulation of the timing of activity offset, for example by exposure to light in the evening (similar to what Santema & Kempenaers, 2023 did in the morning), and manipulation of food availability or of perceived predation risk could shed light on the causality of the association found.

The annual timing of reproduction

The circadian clock not only appears to influence the daily rhythm of activity, but several studies have shown that it is also involved in annual timing of reproduction, i.e. the circannual rhythm (Graham et al., 2017; Helm, 2020; Helm & Visser, 2010; see also Liedvogel et al., 2009; Liedvogel & Sheldon, 2010). Therefore, in **Chapter 2** the correlation between daily timing of activity and annual timing of reproduction was investigated. At first, such a correlation seemed to be present, but upon closer inspection of the data, it was found to be confounded by the date of measurement. Ultimately, by using previously collected data from before the start of the breeding season that were not confounded by date, we were able to show that the correlation between the timing of activity and the start of egg laying was absent in our population (contrary to Graham et al., 2017, but consistent with findings by Helm & Visser, 2010;

Maury et al., 2020; Steinmeyer et al., 2013). Since the photo-inducible phase, (i.e. the diel time frame during which a photoperiodic reaction can be triggered that determines the timing of reproduction; see general introduction), is located at the end of the day, it is possible that the timing of the photo-inducible phase is more strongly related to the timing of activity offset than to that of activity onset, especially since the timing of activity onset and offset were not related to each other (**Chapter 2** and **Chapter 7**). Indeed, in **Chapter 7**, for males, the timing of activity offset measured at the end of winter appeared to be related to the laying date of the first egg. However, the timing of activity offset in males was not only related to laying date, but also to the number of offspring and to male survival, which could again be an indication that the observed relationship could be driven by differences in condition/quality between males. Thus, later laying dates need not to be caused by later timing of the photoinducible phase, but could also be due to poorer condition of the male (Guindre-Parker et al., 2013; Sheppard et al., 2013). Moreover, in **Chapter 2** the correlation in females during the pre-egg laying period was only tested for the timing of activity onset and for active day length. However, when additionally analysing the data and testing the correlation between the laying date of the first egg and the timing of activity offset it was not found to be significant ($\beta = 0.08$, $t = 0.93$, $p = 0.37$). If the timing of daily activity and the timing of annual reproduction were linked at a proximate level, there could be constraints on the independent evolution of both traits, because selection for earlier laying dates could also cause earlier chronotypes. Recently, laying dates have become earlier, due to climate change, which advances the caterpillar peak on which great tits depend to feed their young (Gienapp et al., 2013; Visser et al., 1998). However, given that it is the female who lays the eggs, the laying date of the first egg is probably more strongly determined by her than by the male. As there was no correlation between female emergence time or entry time and the timing of reproduction it seems unlikely that the daily timing of activity is linked to the annual timing of reproduction at a proximate level, in contrast to previous assumptions. Hence, earlier timing of activity onset due to the effects of climate change on the timing of reproduction is unlikely.

Nevertheless, advancing laying dates could put pressure on the timing of activity, as the daylight period is shorter earlier in the year. Since the year 2000, the laying date of the first egg in our population has been advanced by about half a month, leading to a shortening of the daylight period that great tits have at their disposal of about one hour. It is possible that great tits need a minimum amount of time daily to gain enough resources to produce eggs or to raise their young, and the advanced laying dates might force them to be active at times that are risky (i.e. earlier in the morning and later in the evening). As shown in **Chapter 7**, being active later in the evening is related to lower reproductive success and survival (but again, we do not know if this is a causal relationship). Moreover, as shown in **Box 1**, the loss of a known food source led to a longer active day length. Although this was investigated in winter when food is supposedly more limited than in the breeding season, it shows that time can be a scarce resource for great tits and that they extend their day only when necessary (see also Ydenberg, 1984). As it is likely that the caterpillar peak will advance even more in the coming years, we urgently need to know whether the shorter day light periods earlier in the year may at some stage have implications for successful reproduction.

Box 1: The timing of activity depends on food availability

In November 2021, 8 feeders equipped with RFID-readers were placed in the population and were continuously monitored for one month (Beckers, 2022, *unpublished master thesis*). In December, emergence time, entry time and active daylength were measured for all individuals that fed regularly on the feeders and that roosted in nest boxes (N = 18) for 15 days, so that their timing of activity could be measured. During these 15 days food was present from day 1 – 5 and 11 – 15. From day 6 – 10 the feeders were empty. The population was divided into two groups, so the experiment was in anti-synchrony to avoid confounding systematic environmental effects. I.e. for group 2, the experiment started on day 6 of group 1. Emergence times ranged between 72 minutes before sunrise and 9 minutes after sunrise (mean ± SD = -22.9 ± 11.2), entry times between 27 minutes before sunset and 30 minutes after (mean ± SD = 3.2 ± 9.3) and active daylength between 19 minutes shorter than the daylight period and 83 minutes longer (mean ± SD = 26.0 ± 14.8). Active daylength was found to be significantly longer on days when food was absent (LMER: $\beta = 3.45$, df = 239.18, $t = 2.96$, $p = 0.003$) and emergence time was earlier (LMER: $\beta = -2.02$, df = 242.04, $t = -2.92$, $p = 0.004$). Entry times were not significantly affected, but showed a trend that suggested that entry times became later when food was absent (LMER: $\beta = 1.55$, df = 241.01, $t = 1.71$, $p = 0.09$). Timing of activity was thus altered by

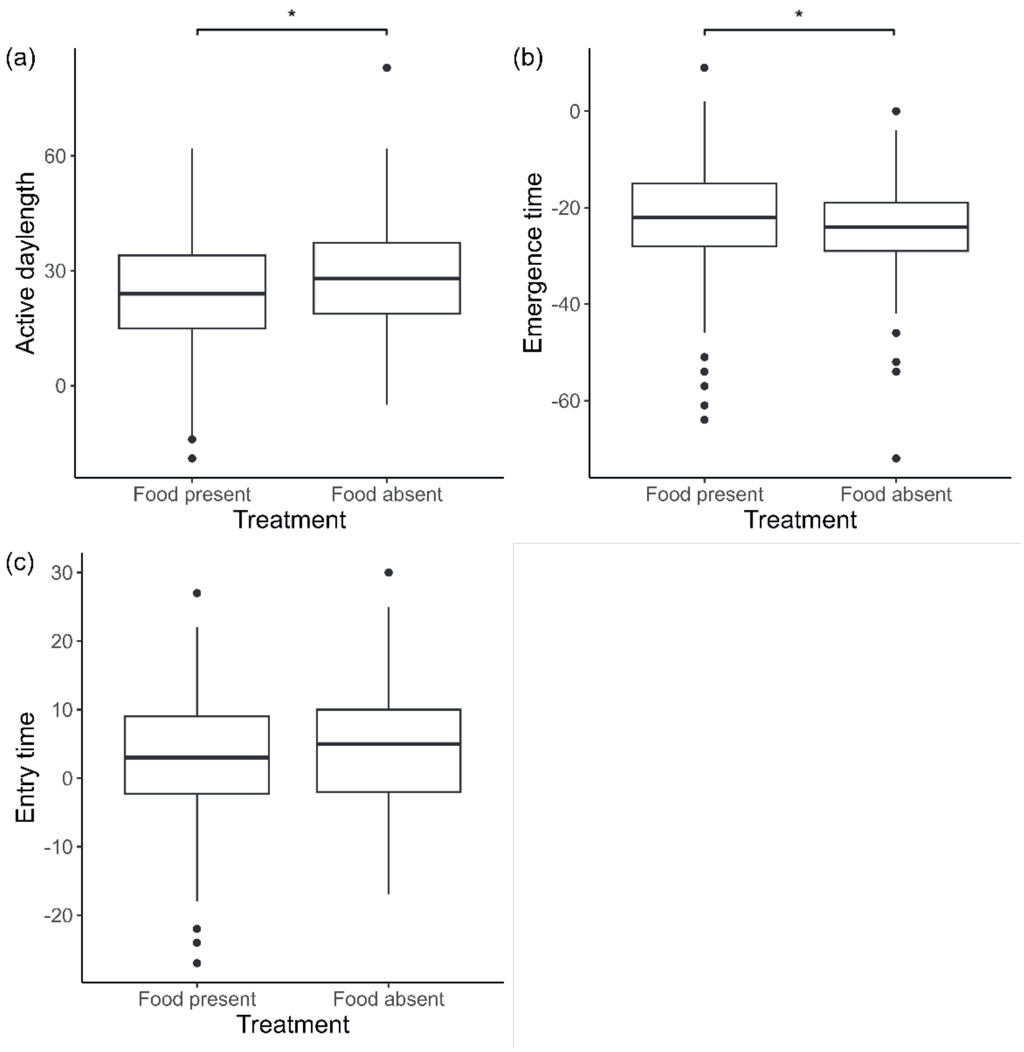


Figure B1: The effect of a sudden absence of a known food source on (a) active daylength (minutes relative to the daylight period), (b) emergence time (minutes relative to sunrise) and (c) entry time (minutes relative to sunset). Based on Beckers (2022).

Animal personality and behavioural syndromes

Within behavioural ecology, there is a growing interest in behavioural traits that consistently differ between individuals. Such traits are referred to as personality traits. Generally, 5 main axes of personality are distinguished: boldness, aggressiveness, neophobia, exploratory behaviour and sociability (Réale et al., 2007). However, many other behaviours may fit in the definition of a personality trait and whether a 5-factor model is sufficient to describe personality in animals is so far unclear (Bell, 2017; Bell et al., 2009). In this thesis I have shown that individuals actually do show consistent differences in the timing of activity (**Chapter 3**), implying that an important criterion of a personality trait is fulfilled. To get a more comprehensive understanding of what personality is and what its functional consequences are, it is therefore recommended that chronotypes are interpreted in a personality framework (see also Bell, 2017).

Furthermore, many behavioural patterns occur in tightly linked suites of traits or "syndromes" (Sih et al., 2004). These behavioural syndromes can be the result of selection favouring particular trait combinations (and hence genetic correlations; e.g. Dingemanse et al., 2007), or constrain the independent evolution of traits (such as in case of pleiotropic effects; e.g. Royauté et al., 2020). In both ways, behavioural syndromes can affect the direction in which behaviours evolve as well as the ability of populations to diverge and respond to selective pressures (Royauté et al., 2020). Yet, thus far chronotypes have not been considered in a syndrome framework, even though correlations with other behavioural traits could be adaptive, or arise from common underlying mechanisms.

As in the case of personality research, studies and concepts of behavioural syndromes have generally not included the timing of activity. Exploration, for example, focusses on spatial use of the environment (i.e. where are they active?), yet differences among individuals in their temporal use of the environment (i.e. when are they active?) matter as well. In this thesis, this was therefore taken into consideration. Here, specifically, the association between aggression and chronotype was investigated. I hypothesized that selection could have favoured this trait combination, because both may play a relevant role in competition for resources (**Chapter 5**). Individuals that are less competitive (i.e. low aggression levels) might advance their activity to forage at lower social densities and avoid competition, thereby increasing the probability of finding sufficient food. A correlation was indeed found between chronotype and aggression, but in the opposite direction than expected: more aggressive females had earlier emergence times. In males, a correlation between aggression and chronotype appeared to be absent (**Box 2**). Thus, a behavioural syndrome that includes aggression and chronotype, seems to be sex-specific. However, it should be noted that for males the period between the chronotype measurements and the aggression tests was relatively long. At current, I lack a functional explanation of the observed trait correlation in females, which renders it unlikely that the result stems from historical selection favouring this trait combinations. Rather the observed syndrome may be due to pleiotropic effects which are supposed to constrain independent trait evolution, explain the occurrence of non-adaptive behaviours, as well as the maintenance of individual variation in behaviour.

Yet before concluding that a shared underlying mechanism drives the correlation between aggression and chronotype, we may have to consider that the correlation may also result from a trade-off between current and future reproduction. Individuals differ in their probability to survive and to breed again in the next year, i.e. their residual reproductive value (RRV), which can be state dependent. Individuals with a low RRV, such as older birds, are expected to increase their investment in current reproduction and may therefore take more risks than individuals with high RRV (Brommer & Class, 2017; Wolf et al., 2007; Wolf & Weissing, 2010), potentially leading to higher aggressiveness to protect the nest and earlier activity onset to increase resource acquisition. Following the predictions from this state-dependent hypothesis, individuals should thus become more aggressive with age, while starting activity earlier. Indeed, older female great tits started activity earlier than yearlings (**Chapter 2, 3 and**

6). However, aggressiveness decreased with age (**Chapter 5**; Thys et al., 2021), making this state-dependent scenario less likely.

Box 2: Aggression is not correlated with chronotype in males.

In the 2023 breeding season, male-male aggression was determined two times (N = 38 males), using territorial intrusion tests following the methods described in **Chapter 5** (*unpublished data*). The time the male spent on the decoy during the test, which was used as a measure of male aggression, was not correlated with the average emergence time, entry time or active daylength (mean centred within days; **Chapter 7**) measured in January (when many males still roost in nest boxes). For the analysis Best Linear Unbiased Predictions (BLUPs) were used, based on a linear mixed effect model with observer and test number as fixed effects and individual as random effect, as both observer (β = 1.33, t = 2.00, p = 0.05) and the test number (β = -0.87, t = -2.03, p = 0.05) tended to affect the time spent on the decoy. In male great tits aggression and chronotype were thus not correlated.

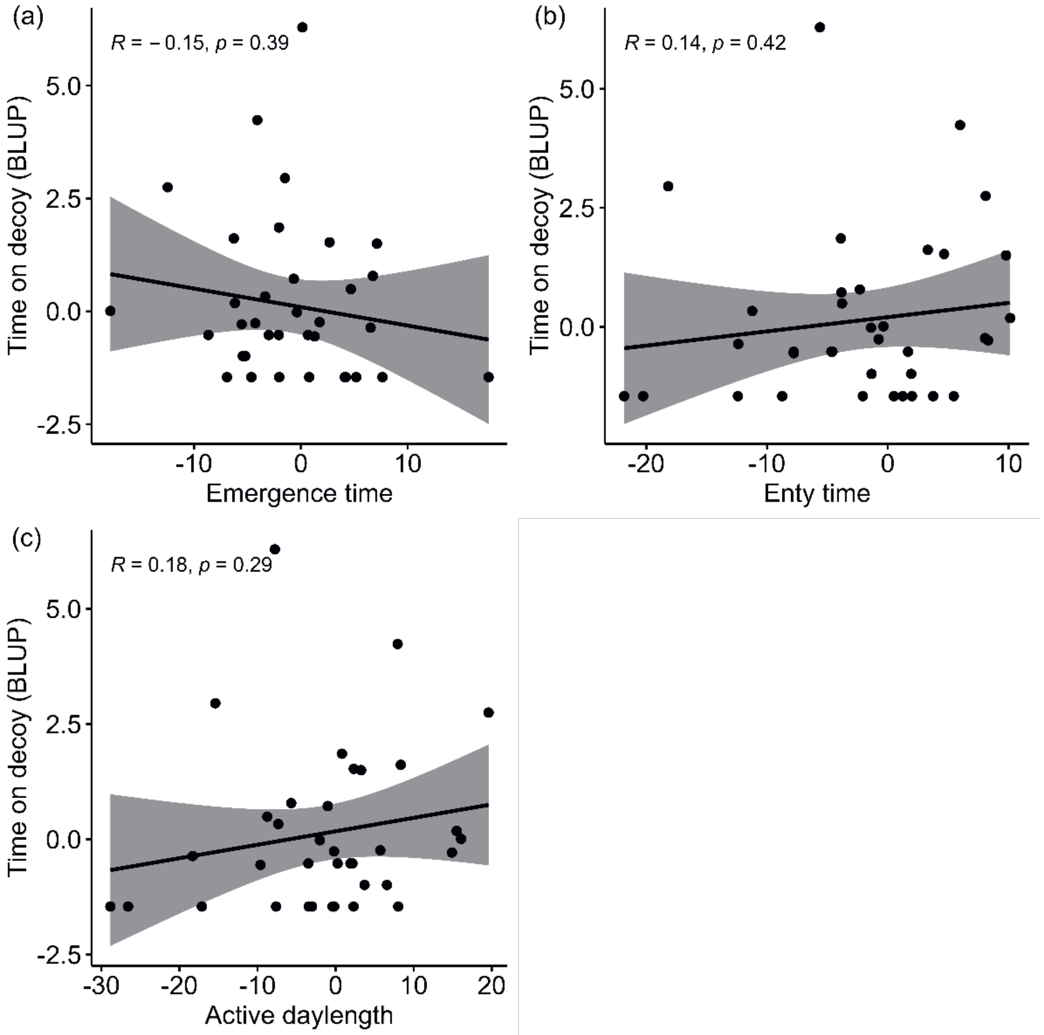


Figure B2: Correlations between (a) emergence time, (b) entry time or (c) active daylength (mean centred within days and averaged within individuals) and the time the male spent on the decoy (BLUP; used as a proxy of aggression) during the territorial intrusion test.

Environmental change

In many habitats, increasing anthropogenic disturbance is disrupting circadian rhythms, for example due to light pollution (Da Silva et al., 2014; de Jong et al., 2017; Kempenaers et al., 2010; Raap et al., 2015; Raap, Pinxten, et al., 2016; Strauß et al., 2024), noise pollution (Dorado-Correa et al., 2016; Fuller et al., 2007; Grunst et al., 2021) and chemical pollution (Thoré et al., 2024). Such a disruption can cause individuals to be active at suboptimal times, but can also cause sleep deprivation. In a suburban population, such as the one in this thesis, where there is great heterogeneity in the level of human-induced disturbance, it is likely that such differences in the level of disturbance contribute to variation in the timing of activity onset and the timing of activity offset. Yet in **Chapter 3**, no effect of nest box location on the timing of activity onset was found and no differences were found in the consistency in timing between females that slept in the same box for several years and females that had moved, despite large differences in, for example, the level of light pollution at small scales (e.g. the levels of light at night in the population ranged between 0.01 and 17.00 lux at the nest box opening and levels of background noise at night ranged between 37.00 and 75.40 dB; **Box 3**; Foppen, 2021). It is, however, possible that individuals actively choose their territory to match their phenotype, so that individuals that are very sensitive to disturbance of their circadian rhythm are not nesting in nest boxes with high levels of disturbance (i.e. matching habitat choice; Bolnick et al., 2003; Edelaar et al., 2008). Although some individuals could thus be sensitive to disturbance, in practice this may only become apparent by experimental manipulation.

The lack of nest box effects in **Chapter 3** may also indicate that the timing of activity onset is little influenced by the environment, as discussed before, and hence also not by anthropogenic stressors present in our population. This presumption was partly confirmed in a study on the effect of light and noise pollution on the timing of the onset of male dawn song, the onset of female calling (i.e. her response to the male while she is still in the nest box in the morning) and female emergence time (**Box 3**). The timing of activity onset in females was only affected by light at very high levels of light pollution. Presumably, at lower levels, the nest box provides sufficient shielding from light pollution for females during the breeding season (Raap et al., 2018), while males, which do not sleep in nest boxes during the breeding season, were affected by light pollution, as they started singing earlier. Since both males and females do not sleep in nest boxes all year, it is likely that light pollution affects both sexes, during different periods of the year, which potentially causes a mismatch in activity timing between partners (e.g. starting dawn song too early). Noise pollution was also related to earlier emergence times, although the effect size was small. Male dawn song, on the other hand, was not affected (**Box 3**). Disruption of circadian rhythms by anthropogenic stressors could potentially result in more extreme phenotypes than occur naturally, and the effects of the timing of activity on fitness could therefore be different or stronger when individuals are exposed to such stressors. This may lead to an evolutionary trap, in which individuals prefer poor quality habitat (in this case on a temporal scale, i.e. starting activity too early), because the cues on which they judge habitat quality (in this case light) no longer correspond to the actual habitat quality due to rapidly changing conditions (Gilbert et al., 2023; Schlaepfer et al., 2002).

Earlier timing of activity onset due to light and noise pollution may also result in shorter sleep duration, but unfortunately, we have no data on the timing of sleep onset for the individuals in **Box 3**, so sleep duration could not be estimated. As shown in **Chapter 4**, sleep deprivation due to light exposure in great tits could lead to impaired cognitive performance. Inhibitory control was lower in individuals that were exposed to artificial light at night than in non-exposed individuals, which may have major implications for fitness. However, so far it is unclear what functions inhibitory control serves in the wild, so whether a decrease in inhibitory control is negative remains to be seen. While low inhibitory control could be an adaptive response in environments where resource availability is unpredictable (Anselme & Güntürkün, 2019; Griffin et al., 2020; but see van Horik et al., 2019), its induction by an artificial stimulus suggests a potential misalignment with natural conditions. Shorter sleep duration could possibly also occur in both males and females during periods throughout the year when they do not sleep in nest boxes and they are likely to be more exposed to light. The research in **Chapter 4** has provided a first hint of the potential consequences of shorter sleep duration, which future research can build on by investigating whether the amount of sleep lost in the wild due to light exposure is sufficient to produce similar effects, whether habituation occurs and whether individuals may exhibit behavioural changes (i.e. seeking a dark sheltered spot) to avoid light exposure. A choice experiment where individuals could choose between a dark roost or an illuminated one already showed that great tits actually chose the illuminated one (Ulgezen et al., 2019), which further stresses the need for investigating the consequences of light pollution and its potential as evolutionary trap.

Box 3: The effects of artificial light at night and noise pollution on the timing of activity onset

During the breeding season in 2021, the timing of the onset of male dawn song, the timing of the onset of female calling inside the nest box in response to the male's song and female emergence time from the nest box were determined using SongMeters (Foppen, 2021, *unpublished master thesis*). After measuring these timing parameters, the levels of light pollution (ALAN) and noise pollution (i.e. maximum level of background noise) at night were determined at the nest box during the egg-laying or incubation period. Female emergence time was affected by ALAN (LMER: β ± CI(95%) = -3.13 [-5.84, -0.42]), but only if one extremely ALAN exposed nest box was included in the analysis. When excluding this nest box, there was no significant effect of light on female emergence time (LMER: β ± CI(95%) = -1.42 [-6.68, 3.85]). The onset of male dawn song was affected by ALAN with and without the most light-polluted nest box included (LMER with: β ± CI(95%) = -8.22 [-13.14, -3.30], LMER without: β ± CI(95%) = -16.02 [-25.04, -6.99]). Noise pollution affected only female emergence time (LMER: β ± CI(95%) = -3.76 [-6.97, -0.54]), but not male dawn song (β ± CI(95%) = 0.28 [-5.60, 6.16]) and the onset of female calling (LMER: β ± CI(95%) = 1.03 [-2.72, 4.78]). Thus, the timing of activity onset was affected by light and noise pollution, but it depended on the intensity of the stressor and not all timing measures were affected in the same way.

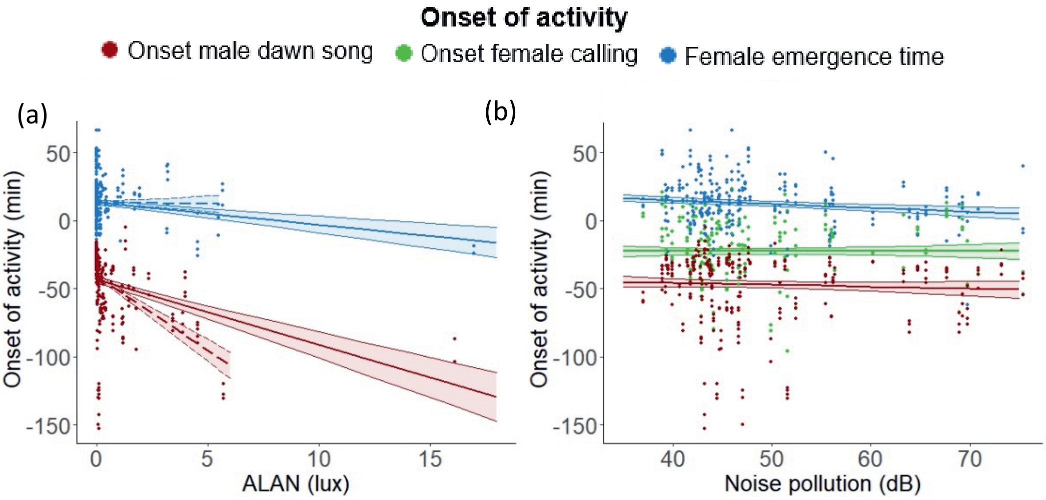


Figure B3: The effect of (a) light pollution (ALAN) and (b) noise pollution on the timing of the onset of male dawn song, the onset of female calling and the female emergence time from the nest box. In Figure A, the solid lines show the effect including the most polluted nest box and the dashed lines show the effect without this box (Foppen, 2021).

This thesis only looked at the effects of direct light on the timing of activity and sleep. However, there is a growing suspicion that indirect light pollution, through reflection against aerosols, also called skyglow, affects circadian rhythms too (Dickerson et al., 2022; Evens et al., 2023; Gaston et al., 2015; Kyba et al., 2011). For example, barnacle geese (*Branta leucopsis*) slept less during cloudy nights, due to increased light reflection (van Hasselt et al., 2021). As light is widely diffused by this reflection, it can be observed even hundreds of kilometres from its source, and 88% of the land area in Europe is under an illuminated sky at night (Falchi et al., 2016). In a highly urbanised environment, as in our population next to a large city like Antwerp, this skyglow is easily observable with the naked eye. Very little is known so far about the effects of skyglow on the timing of activity in birds and whether these effects are similar to those of direct light pollution. As the light intensity of skyglow is relatively low, it is unlikely that it affected emergence times in this thesis, due to the shielding effect of the nest box. However, in the evening, before entering the nest box, individuals may have been exposed which could have affected their entry time. However, skyglow is likely to be less localised than direct light pollution and may therefore affect the entire population simultaneously. Hence, it likely did not contribute to individual variation. Given the ubiquity of this form of light pollution, it is recommended that the effects of skyglow will be included in future research on light pollution and the timing of activity.

Emergence and entry times as a proxy for chronotype

In this thesis the timing of activity onset and, in some chapters, the timing of activity offset were used to capture different aspects of the temporal phenotype, i.e. the chronotype. These were approximated by the emergence time from the nest box in the morning and the entry time into the nest box in the evening (e.g. Graham et al., 2017; Schlicht & Kempenaers, 2020; Womack et al., 2023a). This approach assumes a relationship between the circadian clock and the observed variation among individuals in the timing of activity in the wild. However, given the effects the environment can have on the timing of activity, it is uncertain whether this assumption is valid. A previous study on blackbirds has shown that the endogenous circadian period length (τ) in the lab was linked to the timing of activity onset in the wild for birds living in urban environments (Dominoni et al., 2013). Interestingly, the relationship was context-dependent as there was no link between both traits for birds living in forests. The authors argued that this discrepancy could be due to the strength of the Zeitgeber, which is weakened in urban environments, causing urban birds to follow their circadian rhythms more closely. Forest birds, on the other hand, were more strongly synchronized with their environment and exhibited very little inter-individual variation. However, recently Tomotani et al. (2023) also reported a significant association between the timing of activity onset in the wild and τ for both urban and forest birds. The population studied in this thesis is suburban, so the strength of the Zeitgeber is likely weaker than in forest populations. I also observed a high degree of variation, suggesting that there could be a link between the timing of activity onset and the internal circadian rhythm, but this remains uncertain. Thus, further studies exploring the relationship between the circadian clock and timing of activity in the wild are urgently needed. Intriguingly, in **Chapter 7** the timing of activity onset and offset were not correlated at an among individual level, which is inconsistent with the concept of chronotype in chronobiology that refers to a shift in the overall rhythm relative to an external temporal reference. However, as discussed above, anticipation is likely more important for the correct timing of activity onset than for activity offset as fluctuating environmental variables can be observed to a lesser extent in the morning, resulting in higher plasticity in the timing of activity offset in response to environmental variables. Therefore, it could be argued that the timing of activity onset might have a closer relationship with the circadian clock.

Furthermore, other measures of the timing of activity onset and offset could be relevant as well, such as awakening time and sleep onset (e.g. Stuber et al., 2015). These are more difficult to measure, but in blue tits these highly correlated with emergence time (r = 0.80) and entry time (r = 0.96; Steinmeyer et al., 2013). Thus, the here taken measurements are likely meaningful. In males, studies investigating the timing of activity usually use the start of vocal activity in the morning (e.g. Bruni et al., 2014; Dolan et al., 2007; Poesel et al., 2006), which is likely due to the difficulty of measuring the entry time from the roosting place, as males do not sleep in nest boxes during the breeding season. Therefore, studies including the timing of activity offset in males are scarce, but the association with reproductive success and survival that was observed in **Chapter 7**, shows that the timing of activity offset may be highly relevant. In females, the timing of the start of vocal activity seemed to deviate from emergence time from the nest box (see **Box 3**). However, the onset of vocal activity may be specifically interesting in the context of communication with the partner and this timing may also be driven by the partner (i.e. some females directly reacted to the first song of the male, personal observation), so it may not reflect the female's chronotype. Furthermore, most of my hypothesis were driven by risks and opportunities that can mainly be experienced outside of the nest box, such as predation risk, food availability, competition and cold weather. Overall, using the emergence and entry times from and into the nest box can be considered as meaningful measures here.

Conclusion

This thesis provided new insights into the causes and consequences of individual variation in the timing of activity in wild great tits, as well as the effects of environmental change on daily timing. First, I showed that individual differences in the timing of activity are consistent over long time periods, which improves our ability to judge the evolvability of the trait. Then, I explored the relationship between this variation and reproductive success and survival and reported both were related to the timing of activity offset in males, highlighting the need for studies on daily rhythms to not only consider the timing of activity onset, but also the timing of activity offset. However, understanding fitness consequences of the timing of activity was complicated by the interdependency of the timing of activity and aggression, as such a correlation may hinder the independent evolution of both traits. This suggests that research on the adaptive consequences of individual variation in the timing of activity would benefit from adopting the behavioural syndrome framework and that selection pressures that affect the timing of activity, such as anthropogenic stressors, may have unexpected effects on correlated behaviours. Furthermore, changes in daily activity patterns due to shared proximate mechanisms with the annual timing of reproduction, which is strongly affected by climate change, are unlikely as no evidence for an association between the daily and annual timing was found. However, other anthropogenic stressors, such as artificial light at night and noise pollution, may have important implications for the daily timing of activity in birds. Especially during periods when they do not sleep in nest boxes or when the levels of light pollution are extremely high, their timing and sleep duration may be altered. Although direct evidence for negative effects of light and noise pollution on reproductive success and survival is lacking in my thesis, there are several indications that make it likely that such negative effects are present. For example, sleep deprivation had negative consequences for cognitive performance, and later entry times, for which anthropogenic disturbance can be responsible, were associated with lower reproductive success and survival. Therefore, it seems appropriate here to act on the precautionary principle and adopt stricter policies around permissible light and noise limits, especially given that the effect of light pollution on the emergence time of females was dose-dependent. Previous studies also showed that dose-dependency and the colour of light play a role in how disruptive light and noise pollution are (de Jong et al., 2016; M. L. Grunst et al., 2021; Ulgezen et al., 2019), and such measures could thus make a difference.

Supplementary material

Chapter 2

Table S1: Number of measurements per day relative to clutch initiation during the pre-egg laying and the egg laying phase.

Pre-egg laying phase

	Number of days prior to clutch initiation																
	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
Emergence time	1	3	3	3	3	2	3	5	6	6	4	5	3	6	6	6	3
Entry time	2	2	3	4	3	2	3	5	7	6	5	4	4	6	6	7	4
Active daylength	1	2	3	3	2	2	3	5	6	6	4	4	3	6	6	6	3

Egg laying phase

	Number of days after clutch initiation							
	0	1	2	3	4	5	6	7
Emergence time	62	94	102	87	74	39	4	3
Entry time	93	101	85	76	45	9	2	0
Active daylength	55	89	73	74	39	8	2	0

Table S2: Results from linear mixed effects models for testing the influence of date on emergence time, entry time and active daylength (in minutes relative to sunrise, sunset and the period between sunrise and sunset respectively) during the pre-egg laying phase. Estimates in bold are statistically significant (P < 0.05).

Dependent variable	*Fixed effects*	β	SE	t	df	*P*
Emergence time	Date	2.24	1.61	1.39	53.52	0.17
	Days prior to egg laying	-0.33	0.57	-0.57	17.63	0.57
	Age	-4.72	3.63	-1.30	17.11	0.21
	$T_{sunrise}$	-0.02	0.78	-0.02	42.36	0.98
Entry time	Date	3.01	4.16	0.72	53.97	0.47
	Days prior to egg laying	0.89	0.56	1.59	18.89	0.13
	Age	0.60	3.64	0.16	17.89	0.87
	T_{sunset}	**3.50**	**1.28**	**2.73**	**48.61**	**<0.01**
Active daylength	Date	-0.65	3.58	-0.18	46.14	0.86
	Days prior to egg laying	0.69	0.86	0.80	16.29	0.44
	Age	5.10	5.26	0.97	16.20	0.35
	T_{max}	**4.35**	**1.08**	**4.02**	**38.04**	**<0.001**

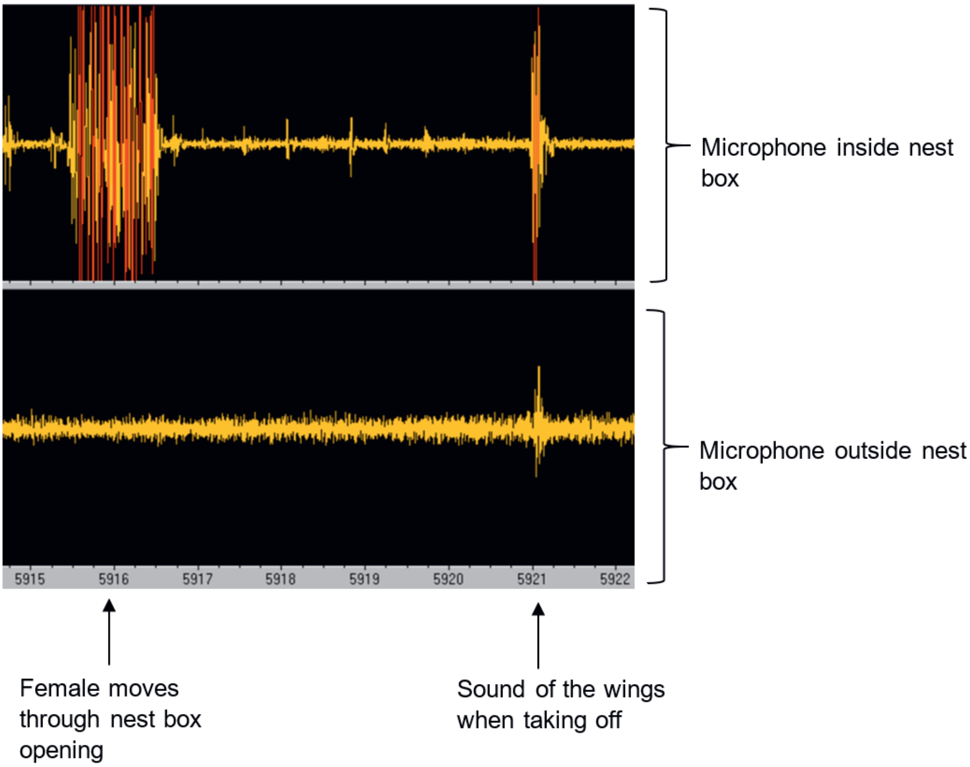


Figure S1: audio graph created by Avisoft SASLab Pro 5.2.14, illustrating the emergence of a female from the nest box.

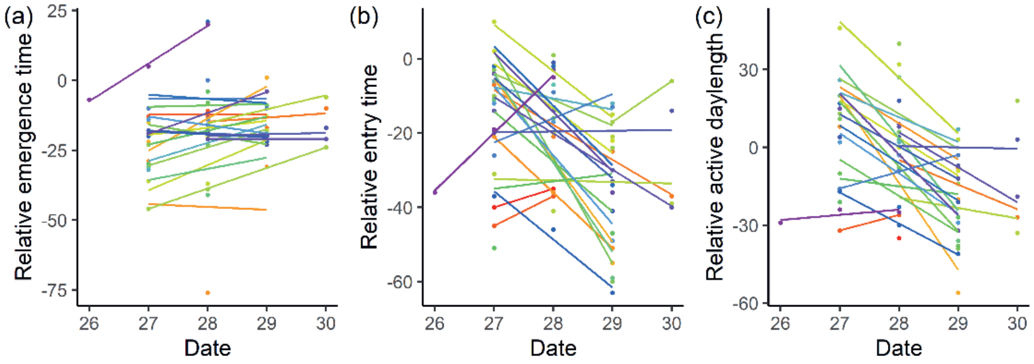


Figure S2: *Activity patterns in female great tits during the pre-egg laying period: a) emergence times relative to sunrise, b) entry times relative to sunset and c) active daylength in minutes relative to the period between sunrise and sunset. All individuals have separate regression lines (individuals can be distinguished by colour). Date starts as a count from April 1 (= 1).*

Chapter 3

Table S1: Results from linear mixed effects model which included only females that moved between nest boxes, with emergence time (in minutes relative to sunrise) as response variable. Date and temperature (T) at sunrise were mean-centred within years. 95% confidence intervals are calculated with parametric bootstrapping and are shown between brackets.

Fixed effect	β	T	Lower 95% CI	Upper 95% CI
Poly(Centred Date)1	112.60	4.58	85.1	140.3
Poly(Centred Date)2	61.95	2.87	45.57	105.70
Centred T sunrise	-0.27	-1.24	-0.63	0.20
Year 2019	0.66	0.17	-2.93	4.27
Year 2020	-3.37	-0.83	-6.91	-0.32
Year 2021	9.82	2.02	5.50	13.06
Older/Yearling	-6.28	-2.04	-9.07	-4.02
Random effect	σ^2		Lower 95% CI	Upper 95% CI
FemaleID	75.49		50.81	104.70
FemaleID_Year	101.50		93.4	151.1
Residual	83.91		69.93	98.48

Table S2: Results from linear mixed effects model which included only females that did not move between nest boxes, with emergence time (in minutes relative to sunrise) as response variable. Date and temperature (T) at sunrise were mean-centred within years. 95% confidence intervals are calculated with parametric bootstrapping and are shown between brackets. Note: the estimate for FemaleID_Year falls outside of the confidence interval. However, using the estimate for the calculation of the repeatability gives very similar results to using the upper limit of the confidence interval (with estimate R= 0.29 [0.20, 0.36]; with upper limit of the confidence interval R = 0.25 [0.19, 0.34]). Thus, this does not pose problems for the interpretation of the results.

Fixed effect	β	T	Lower 95% CI	Upper 95% CI
Poly(Centred Date)1	97.57	3.99	88.45	142.77
Poly(Centred Date)2	44.40	1.97	15.29	69.45
Centred T sunrise	-0.25	-1.29	-0.40	0.33
Year 2019	0.47	0.19	-2.78	3.52
Year 2020	-2.46	-0.88	-5.94	0.89
Year 2021	3.95	1.16	-0.20	8.12
Older/Yearling	-3.82	-1.79	-5.79	-0.54
Random effect	σ^2		Lower 95% CI	Upper 95% CI
FemaleID	68.11		51.18	90.32
FemaleID_Year	43.74		45.83	80.04
Residual	119.30		103.4	135.0

Chapter 4

Table S1: Results from generalised linear models with negative binomial distributions, with exploration score as dependent variable (n = 52). All models included group (ALAN-exposed/non-exposed) and population (Fort7/CDE) as fixed factors. All fixed effects shown in this table were tested in separate models. As population and test date were correlated test date was not included in this table.

Fixed effect	β	z-value	P-value
Sex[a]	0.11	0.31	0.76
Age[b]	0.14	0.48	0.64
Start Time Test	0.01	0.41	0.68
Sleep Duration	0.01	0.59	0.56
Emergence Time	0.01	0.66	0.51
Entry Time	0.00	-0.07	0.95
Group x Population[c]	0.51	0.71	0.48
Group x Sleep Duration	0.02	1.15	0.25
Group x Emergence Time	0.01	0.64	0.52
Group x Entry Time	-0.02	-0.82	0.41

[a] Female is used as reference for the sex effect

[b] Birds older than one year are used as reference for the age effect

[c] CDE is used as reference for the population effect

Table S2: AICc values and degrees of freedom for all the models tested with exploration score as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df
Group	367.59	3
Group + Sex	369.84	4
Group + Age	369.43	4
Group + Population	**364.04**	**4**
Group + Date	366.37	4
Group + Start Time Test	366.06	4
Group + Sleep Duration	369.32	4
Group + Emergence Time	369.19	4
Group + Entry Time	369.92	4
Group + Population + Sex	366.40	5
Group + Population + Age	366.27	5
Group + Population + Date	366.48	5
Group + Population + Start Time Test	366.30	5
Group + Population + Sleep Duration	366.19	5
Group + Population + Emergence Time	366.09	5
Group + Population + Entry Time	366.48	5
Group x Population	365.99	5
Group + Population + Group x Sleep Duration	367.58	6
Group + Population + Group x Emergence Time	368.26	6
Group + Population + Group x Entry Time	368.22	6

Table S3: Results from generalised linear models with negative binomial distributions, with the number of scans in the vigilance test as dependent variable (n = 50). All models included group (ALAN-exposed/non-exposed) and the start time of the test as fixed effects. All fixed effects shown in this table were tested in separate models.

Fixed effect	β	z-value	P-value
Sex[a]	-0.07	-0.40	0.69
Age[b]	-0.16	-1.11	0.27
Date	0.02	1.31	0.19
Population[c]	-0.10	-0.62	0.54
Sleep Duration	0.00	0.21	0.84
Emergence Time	0.00	-0.81	0.42
Entry Time	-0.01	-1.34	0.17
Group x Population	-0.32	-0.96	0.34
Group x Sleep Duration	0.00	0.40	0.69
Group x Emergence Time	0.01	0.77	0.44
Group x Entry Time	0.01	0.59	0.56

[a] Female is used as reference for the sex effect

[b] Birds older than one year are used as reference for the age effect

[c] CDE is used as reference for the population effect

Table S4: AICc values and degrees of freedom for all the models tested with the number of scans during the vigilance test as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df
Group	335.74	3
Group + Sex	337.76	4
Group + Age	338.06	4
Group + Population	337.82	4
Group + Date	334.91	4
Group + Start Time Test	**317.10**	**4**
Group + Sleep Duration	338.11	4
Group + Emergence Time	338.05	4
Group + Entry Time	338.05	4
Group + Start Time Test + Sex	319.41	5
Group + Start Time Test + Age	318.35	5
Group + Start Time Test + Population	319.30	5
Group + Start Time Test + Date	317.86	5
Group + Start Time Test + Sleep Duration	319.53	5
Group + Start Time Test + Emergence Time	318.91	5
Group + Start Time Test + Entry Time	317.77	5
Group x Population	320.88	6
Group x Sleep Duration	321.95	6
Group x Emergence Time	320.91	6
Group x Entry Time	320.04	6

Table S5: Results from linear models with the percentage of time spent on scanning in the vigilance test as dependent variable (n = 50). All models included group (ALAN-exposed/non-exposed) and the start time of the test as fixed effects. All fixed effects shown in this table were tested in separate models.

Fixed effect	β	z-value	P-value
Sex[a]	2.88	0.66	0.52
Age[b]	-0.86	-0.23	0.82
Date	0.21	0.57	0.57
Population[c]	-0.05	-0.01	0.99
Sleep Duration	-0.01	-0.11	0.92
Emergence Time	-0.08	-0.58	0.57
Entry Time	-0.09	-0.54	0.59
Group x Population	1.99	0.22	0.83
Group x Sleep Duration	0.18	0.80	0.43
Group x Emergence Time	0.23	0.82	0.42
Group x Entry Time	-0.05	-0.14	0.89

[a] Female is used as reference for the sex effect

[b] Birds older than one year are used as reference for the age effect

[c] CDE is used as reference for the population effect

Table S6: AICc values and degrees of freedom for all the models tested with the number the percentage of time spent on scanning during the vigilance test as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df
Group	426.72	3
Group + Sex	429.07	4
Group + Age	428.96	4
Group + Population	429.07	4
Group + Date	427.47	4
Group + Start Time Test	**403.38**	**4**
Group + Sleep Duration	428.98	4
Group + Emergence Time	429.06	4
Group + Entry Time	428.98	4
Group + Start Time Test + Sex	405.39	5
Group + Start Time Test + Age	405.80	5
Group + Start Time Test + Population	405.86	5
Group + Start Time Test + Date	405.51	5
Group + Start Time Test + Sleep Duration	405.84	5
Group + Start Time Test + Emergence Time	405.50	5
Group + Start Time Test + Entry Time	405.54	5
Group x Population	408.39	6
Group x Sleep Duration	407.72	6
Group x Emergence Time	407.34	6
Group x Entry Time	408.11	6

Table S7: Results from generalised linear models with a binomial distribution, with the proportion of successful trials in the detour reach task as dependent variable (n = 35). All models included group (ALAN-exposed/non-exposed), the population (Fort7/CDE) and the entry time as fixed effects. All fixed effects shown in this table were tested in separate models. As entry time, emergence time and sleep duration were correlated, emergence time and sleep duration were not included in this table. Population and test date were also correlated, so test date was not included in this table.

Fixed effect	β	z-value	P-value
Sex[a]	-0.98	-1.93	0.05
Age[b]	0.73	2.05	0.04
Number of Worms Eaten during Habituation	-0.33	-0.70	0.48
Group x Population[c]	1.30	1.18	0.24
Group x Entry Time	-0.08	-1.71	0.09

Table S8: AICc values and degrees of freedom for all the models tested with the proportion of successful trials during the detour reach task as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df
Group	146.87	2
Group + Sex	149.21	3
Group + Age	144.94	3
Group + Population	126.51	3
Group + Date	127.36	3
Group + Number of Worms Eaten during Habituation	147.91	3
Group + Sleep Duration	148.23	3
Group + Emergence Time	145.92	3
Group + Entry Time	128.85	3
Group + Population + Sex	128.86	4
Group + Population + Age	127.07	4
Group + Population + Date	127.05	4
Group + Population + Number of Worms Eaten during Habituation	127.81	4
Group + Population + Sleep Duration	128.93	4
Group + Population + Emergence Time	124.89	4
Group + Population + Entry Time	**114.87**	**4**
Group + Population + Entry Time + Sex	113.51	5
Group + Population + Entry Time + Age	113.26	5
Group + Population + Entry Time + Date	117.37	5
Group + Population + Entry Time + Number of Worms Eaten during Habituation	117.10	5
Group + Population + Entry Time + Group x Population	116.13	5
Group + Population + Group x Entry Time	114.49	5

Table S9: Results from a generalised linear mixed model with a binomial distribution, with the score (success/fail) in the detour reach task as dependent variable ($n_{ALAN\text{-}exposed}$ = 16, $n_{non\text{-}exposed}$ = 19; n_{CDE} = 27, n_{Fort7} = 8).

Fixed effect	β	SE	t-value	P-value
Group *Non-exposed*[a]	0.92	0.43	2.15	0.032
Population *Fort7*[b]	-2.02	0.60	-3.35	<0.001
Entry time	0.07	0.02	3.04	<0.01

[a]The ALAN-exposed group is used as reference for the group effect

[b]CDE is used as reference for the population effect

Table S10: Results from generalised linear mixed models with a binomial distribution, with the score (success/fail) in the detour reach task as dependent variable (n = 35). All models included group (ALAN-exposed/non-exposed), the population (Fort7/CDE) and the entry time as fixed effects. All fixed effects shown in this table were tested in separate models. As entry time, emergence time and sleep duration were correlated, emergence time and sleep duration were not included in this table. Population and test date were also correlated, so test date was not included in this table.

Fixed effect	β	z-value	P-value
Sex[a]	-1.06	-1.75	0.08
Age[b]	0.74	1.75	0.08
Trial	-0.05	-0.66	0.51
Number of Worms Eaten during Habituation	-0.24	-0.42	0.67
Group x Population[c]	1.74	1.29	0.20
Group x Trial	0.03	0.16	0.88
Group x Entry Time	-0.09	-1.64	0.10

[a] Female is used as reference for the sex effect

[b] Birds older than one year are used as reference for the age effect

[c] CDE is used as reference for the population effect

Table S11: AICc values and degrees of freedom for all the models tested with the score during the detour reach task as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df	Comments
Group	246.26	3	
Group + Sex	248.26	4	Model failed to converge
Group + Age	246.49	4	
Group + Population	235.81	4	
Group + Date	236.27	4	
Group + Trial	247.87	4	
Group + Number of Worms Eaten during Habituation	247.49	4	
Group + Sleep Duration	247.93	4	
Group + Emergence Time	246.69	4	
Group + Entry Time	238.34	4	
Group + Population + Sex	237.78	5	
Group + Population + Age	236.81	5	
Group + Population + Date	236.69	5	
Group + Population + Trial	237.38	5	
Group + Population + Number of Worms Eaten during Habituation	237.06	5	
Group + Population + Sleep Duration	237.81	5	
Group + Population + Emergence Time	235.53	5	
Group + Population + Entry Time	**228.86**	**5**	
Group + Population + Entry Time + Sex	227.78	6	
Group + Population + Entry Time + Age	228.02	6	
Group + Population + Entry Time + Date	230.82		
Group + Population + Entry Time + Trial	230.55	6	
Group + Population + Entry Time + Number of Worms Eaten during Habituation	230.81	6	
Group + Population + Entry Time + Group x Population	229.15	6	
Group + Population + Group x Entry Time	228.08	6	
Group + Population + Entry Time + Group x Trial	232.67	7	

Table S12: Results from linear models, with the number of pecks in the detour reach task as dependent variable (log-transformed; n = 29). All models included group (ALAN-exposed/non-exposed) and the entry time as fixed effects. All fixed effects shown in this table were tested in separate models. As entry time, emergence time and sleep duration were correlated, emergence time and sleep duration were not included in this table.

Fixed effect	β	z-value	P-value
Sex[a]	0.11	0.19	0.85
Age[b]	-0.16	-0.37	0.72
Date	-0.02	-0.54	0.59
Population[c]	-0.02	-0.04	0.97
Number of Worms Eaten during Habituation	-0.22	-0.49	0.63
Group x Population	0.62	0.64	0.53
Group x Entry Time	-0.04	-0.80	0.43

[a] Female is used as reference for the sex effect

[b] Birds older than one year are used as reference for the age effect

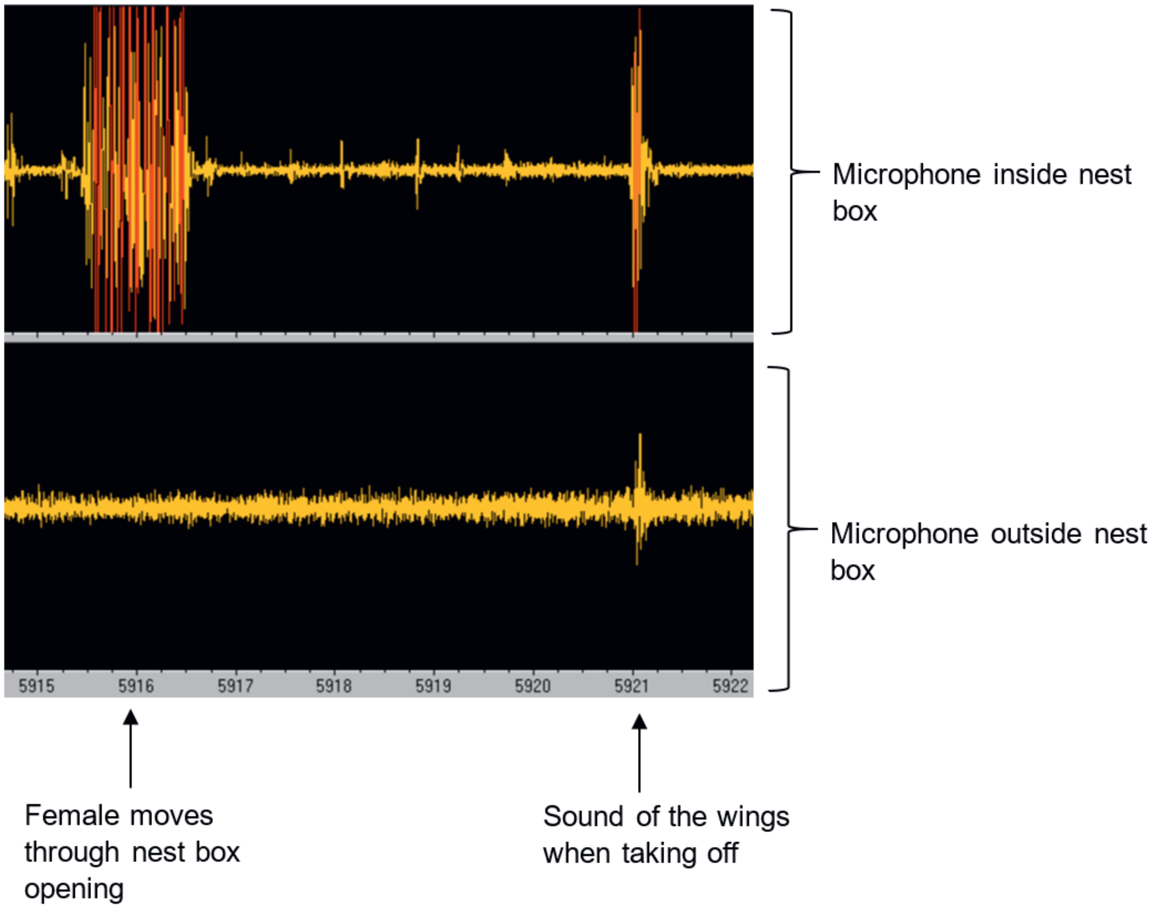
[c] CDE is used as reference for the population effect

Table S13: AICc values and degrees of freedom for all the models tested with the score during the detour reach task as dependent variable. The model in bold is the selected model.

Fixed effect	AICc	df
Group	96.14	3
Group + Sex	98.16	4
Group + Age	98.71	4
Group + Population	98.84	4
Group + Date	97.90	4
Group + Number of Worms Eaten during Habituation	98.20	4
Group + Sleep Duration	96.63	4
Group + Emergence Time	98.80	4
Group + Entry Time	**93.45**	**4**
Group + Entry Time + Sex	96.35	5
Group + Entry Time + Age	96.23	5
Group + Entry Time + Population	96.39	5
Group + Entry Time + Date	96.04	5
Group + Entry Time + Number of Worms Eaten during Habituation	96.11	5
Group x Entry Time	95.66	5
Group + Entry Time + Group x Population	99.10	6

Chapter 5

Figure S1: audio graph created by Avisoft SASLab Pro 5.2.14, illustrating the emergence of a female from the nest box.



Prior specification for the multivariate mixed model with all aggression parameters

```
prior1 = list(R = list(V = diag(4), nu = 0.004),
               G = list(G1 = list(V = diag(4), nu = 4, alpha.mu = rep(0,4), alpha.V = diag(25^2,4,4,4))))
```

Prior specification for the multivariate mixed model with emergence time and time on the decoy

```
prior2 = list(R = list(V = diag(2), nu = 0.002),
               G = list(G1 = list(V = diag(2), nu = 2, alpha.mu = rep(0,2), alpha.V = diag(25^2,2))))
```

Table S1: descriptive statistics on the behavioural measurements during the territorial intrusion test and on emergence time.

	Mean	SD	Min	Max
Approach Distance	0.72	1.72	0	15
No. Calls	20.2	31.1	0	158
No. Attacks	3.0	7.7	0	61
Time Decoy	38.8	64.5	0	288
Emergence Time	8.4	16.9	-54	91

Chapter 6

```
priorAnnual = list(R = list(V = diag(2), nu = 0.002),
                   G = list(G1 = list(V = diag(2), nu = 2,
                                      alpha.mu = rep(0,2),
                                      alpha.V = diag(25^2,2,2))))


priorLifeTime = list(R = list(V = diag(c(1,0.0001),2), nu = 1.002, fix = 2),
                     G = list(G1 = list(V = diag(2), nu = 2,
                                        alpha.mu = rep(0,2),
                                        alpha.V = diag(25^2,2,2))))
```

Table S1: Results from two bivariate mixed model with emergence time from the nest box (in minutes relative to sunrise) as first response variable and respectively the relative annual number of eggs (only first clutches included) and the relative annual number of fledglings (only first clutches included) as second response variables. The results for emergence times are not shown. Estimates of fixed (β) and random (σ²) components are shown with 95% credibility intervals (CrI) and fixed effects that found strong support are presented in bold.

	Relative annual number of eggs (only first clutches)	**Relative annual number of fledglings (only first clutches)**
Fixed effects		
Intercept	**0.993 (0.942, 1.038)**	**1.188 (1.066, 1.308)**
Date[b]	-	-
Date 2[b]	-	-
T at sunrise[c]	-	-
Year 2019[a]	0.032 (-0.019, 0.077)	**-0.015 (-0.131, 0.120)**
Year 2020[a]	-0.039 (-0.091, 0.014)	-0.363 (-0.502, -0.223)
Year 2021[a]	-0.044 (-0.112, 0.022)	-0.326 (-0.486, -0.147)
Age	0.005 (-0.017, 0.028)	-0.025 (-0.079, 0.029)
Clutch initiation date[d]	**-0.005 (-0.008, -0.001)**	-0.009 (-0.019, 0.001)
Random effects		
FemaleID	0.017 (0.010, 0.024)	0.044 (0, 0.092)
Residual	0.023 (0.018, 0.028)	0.215 (0.165, 0.274)

Table S2: Covariances and correlations (with 95% credible intervals) regarding emergence time and the absolute deviation from the population mean of emergence time from the nest box in relation to the annual number of eggs and fledglings (only first clutches included) at the among- and within-individual level.

Response variables	**Covariance among individuals**	**Correlation among individuals**	**Covariance within individuals**	**Correlation within individuals**
Emergence time & number of eggs (annual)	0.001 (-0.017, 0.020)	0.009 (-0.191, 0.206)	0.002 (-0.011, 0.014)	0.019 (-0.109, 0.130)
Emergence time & number of fledglings (annual)	0.00 (-0.038, 0.047)	-0.007 (-0.500, 0.430)	0.022 (-0.014, 0.060)	0.068 (-0.048, 0.181)
Emergence time (deviation from population mean) & number of eggs (annual)	0.000 (-0.018, 0.017)	0.003 (-0.225, 0.213)	0.008 (-0.008, 0.023)	0.067 (-0.055, 0.194)
Emergence time (deviation from population mean) & number of fledglings (annual)	-0.022 (-0.063, 0.017)	-0.214 (-0.713, 0.182)	-0.003 (-0.045, 0.044)	-0.008 (-0.125, 0.108)

Chapter 7

```
PriorBivariateModels = list(R = list(V = diag(2), nu = 0.002),
                            G = list(G1 = list(V = diag(2), nu = 2,
                                               alpha.mu = rep(0,2),
                                               alpha.V = diag(25^2,2))))


PriorUnivariateModels = list(R = list(V = diag(1), nu = 0.002))
```

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Acknowledgements

Als eerste zou ik graag Wendt en Marcel willen bedanken voor de kans om te promoveren in jullie onderzoeksgroep en voor alles wat ik van jullie heb mogen leren. Ik heb een hele fijne tijd gehad onder jullie supervisie en heb altijd het gevoel gehad dat jullie erop vertrouwden dat het goed zou komen, wat mij weer veel vertrouwen en motivatie gaf. Ook wil ik jullie bedanken dat jullie er waren op momenten dat het nodig was, bijvoorbeeld als een experiment misliep, waardoor ik mij nooit alleen voelde.

Ook wil ik mijn PhD-comité bedanken, Raoul, Erik, Alizée, Lotte en Kamiel, voor het lezen van mijn thesis en het van feedback voorzien, waardoor de kwaliteit zeker verbeterd is.

Peter en Geert, toen ik begon met mijn PhD was er door Covid-19 vrijwel niemand op de campus behalve wij drieën. Dank jullie wel om mij hier toch thuis te doen voelen! Ik ben jullie ook super dankbaar voor alle hulp die ik van jullie gekregen heb met het veldwerk. Geert, jij hebt altijd ingenieuze oplossingen voor technische problemen, die alles zoveel makkelijker maakten. Peter, nachtcontroles zijn nooit mijn favoriete bezigheid geworden, maar met jou vond ik het toch altijd gezellig. Ook nog eens bedankt voor het zagen van mijn tafeltjes!

Bert, ik wil jou graag bedanken voor je behulpzaamheid en voor hoe genereus jij bent in het delen van jouw kennis. Ik heb super veel van je geleerd!

Ook wil ik graag Clara, Robin en Alejandro bedanken. Jullie hebben mijn PhD-periode zo opgevrolijkt! Dank jullie wel voor alle klimsessies, therapeutische theedrinkpauzes en yoga tijdens de lunch. Ik vond het heel fijn om mijn PhD-tijd met jullie te mogen delen en ben heel blij dat ik jullie heb leren kennen.

Daarnaast wil ik graag alle BECO-leden bedanken, Reyes, Mélibée, Nicky, Jonas, Daan, Valentin, Tim, Jolien, Miguel, Richard en Bram, voor alle fijne lunchpauzes en group-meetings.

Bovendien ben ik alle studenten die geholpen hebben met het veldwerk enorm dankbaar: Kevin, Lore, Emma en Lauren. Jullie enthousiasme was heel fijn om te zien!

Josie, dankjewel voor jouw organisatorisch talent; zonder jou zou er een hoop in de soep lopen!

Ingrid, dankjewel dat ik altijd bij jou mocht komen slapen en voor alle vegan recepten die we samen hebben uitgeprobeerd.

Mijn dankbaarheid gaat ook uit naar mijn familie en vrienden, die naar mij geluisterd hebben als ik dat nodig had, maar vooral ook juist voor alle fijne afleiding van de PhD die ze mij gegeven hebben.

Als laatst wil ik Matthias bedanken, voor je eindeloze steun en vertrouwen, voor al die honderden grafieken en problemen die ik je heb laten zien en waarnaar je hebt geluisterd, maar bovenal voor hoe fijn het is om na het werk bij jou thuis te komen.