The development and maintenance of migratory timing programs in a songbird

by

Saeedeh Bani Assadi

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba

in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences

University of Manitoba

Winnipeg

Copyright © 2021 by Saeedeh Bani Assadi

Abstract

Advancing spring phenology due to climate change can result in different behavioural responses in long-distance migratory birds, such advancement in their spring arrival date. However, the degree to which timing is flexible to environmental change and the underlying mechanisms require further investigation. To investigate timekeeping systems in free-living long-distance migratory birds (purple martin *Progne subis*) in a natural ecosystem, this thesis used the 'wild clock' approach, which is the combination of chronobiology and ecology. Birds may flexibly respond to advancing springs if earlier first egg dates expose hatched birds to different environmental cues, such as photoperiod, which they may use to synchronize their internal clock time (ontogenetic effect). I found that nest timing (first egg date) was an influential factor on the post-breeding movement timing (fledge date and colony departure date) in a wild population of purple martins. I used an experimental approach to further explore the phenotypic plasticity of young purple martins to photoperiod experienced in the nest. With a simulated, early photoperiod I found that exposed nestlings had a longer nesting period and later fledge and autumn departure dates than birds that experienced natural day length. I also found that an anthropogenic light at night (ALAN) treatment changed the timing of post-breeding movements, where nestlings exposed to white light had higher weight and later colony departure date than young who experienced green light and natural darkness. Lastly, I investigated the impact of aging on timing. Using data for 1-5 year old birds, I found that spring migration timing and the timing of nesting advanced as birds age, which may reflect the effects of experience or that optimal time is under different time selection pressures as birds age. Overall, this study contributes to our understanding of the synchronization of internal clock time during nestling development with one of the most important *zeitgebers*, photoperiod, and its carry-over effects on migration timing as well as the impact of age on

Ш

migratory strategies. Future research could investigate whether timing developed in the nest to photoperiod continues into adulthood providing further insight into climate change impacts on migration timing.

Acknowledgements

First and foremost, I would like to express my deepest appreciation to my supervisor, Kevin Fraser for being endlessly positive, patient and supportive all along with my study and for opening my mind to think differently about ecological studies. I learned a lot from you and thanks for giving me this opportunity to follow my dream. My sincere thank also goes to my committee members Colin Garroway, Saman Muthukumarana and Gail Davoren for their input and guidance along this route. I would like to thank Emily Mckinnon for her help when I started my study at the University of Manitoba. I also thank the past and present Fraser lab members, especially Alicia Korpach, Evelien de Greef, Leanne Neufeld, Maryse Gagné, Ashley Pylypowich, and Christie Lavallee for their friendship and all the supports during my study and fieldwork. I am grateful to my friend, Chloe Schmidt, for her friendship and for being generous in sharing her knowledge.

I thank Kristian Melo for designing, building and programming the LED light apparatuses. I also want to appreciate the purple martin colony managers Alan Enns, Paul and Maxine Clifton for their patience and assistance with this research.

I am particularly grateful to my family, my lovely parents and my dearest sister and brother back in Iran, for their close support and cheering me up from a distance through this journey.

Last but certainly not least, my special thank goes to my best friend, my soulmate, my love of life, Mohammad, for always being there for me and for believing in me. Thanks for your love, your patient, your support over the past years.

IV

Table of Content

Chapter 1: Introduction	9
General information	9
Purple martin as a study system	
Objectives	14
References	
Chapter 2: Does hatch date set the clock? Timing of post-fledging movem	ents for
families of a colonially breeding, long-distance migratory songbird	
Abstract	
Introduction	
Methods	
Statistical Analysis	
Results	
Discussion	
Conclusion	
Acknowledgment	
References	
Chapter 3: Experimental manipulation of photoperiod influences migrati	on timing in a
wild, long-distance migratory songbird	
Abstract	
Introduction	
Methods	
Data Analysis	
Results	
Discussion	
Conclusion	58
Acknowledgment	58
References	60
Chapter 4: The Influence of Different Light Wavelengths of Anthropoger Night on Negling Development and the Timing of Past fladge Meyement	nic Light at
Migratory Songbird	5 III a 69
Abstract	
Introduction	
Materials and Methods	
Data Analysis	

Results	77
Discussion	79
Conclusion	84
Acknowledgment	85
References	86
Chapter 5: As birds age the timing of migration advances and stopover duration decreases in a long-distance migratory songbird	95
Abstract	96
Introduction	97
Methods	99
Data Analysis	101
Results	104
Discussion	105
Conclusion	110
Acknowledgment	110
References	111
Chapter 6: Conclusion	129
References	133
Appendix	134
Supplementary information of chapter 2	134
Supplementary information of chapter 3	141
Supplementary information of chapter 4	144
Supplementary information of chapter 5	150

List of tables

Table 2.1 : LMM analysis to explain variation in timing of fledge date and colony departuredate of juveniles and adult purple martins. The fixed effects were first egg date, number ofnestmates, age of parents, weight of nestlings and fat score. The cavity ID was the randomeffect. This table shows the best supported candidate models for each. The full set ofcandidate models are presented in supplementary tables 2.1-2.2.41
Table 3.1: Top linear mixed-effects factors that explain variation in timing of fledge date and colony departure date of juveniles, as well as nesting duration and duration at the colony post-fledge. The variables we tested include the treatment, first egg date, and the number of nestmates. The global model of fledge date and nesting period also included individual fat score and weight at the time of radio-tagging
Table 4.1: The best models of the linear mixed-effects analysis of effects of ALAN, weight, first egg date, nestmate numbers on nesting period, fledge date, and effects of ALAN, first egg date, nestmate numbers on duration of staying at the colony and colony departure date. The cavity ID is considered as a random effect
Table 5.1: Top linear mixed models (LMMs) and linear model (LM) for stopover duration and numbers in fall and spring migration
Table 5.2: Top generalized linear models (GLM) with negative binomial distribution, for total stopover duration before and after crossing of the Gulf of Mexico in fall and spring migration
Table 5.3: Top linear model (LM) for flight ration (flight days/stopover days) in fall and spring migration
Table 5.4: Top linear mixed models (LMM) for total fall migration timing
Table 5.5: Top linear mixed models (LMM) for total spring migration timing
Table 5.6: Top mixed models (LMM) for influential factors on nest timing
Table 5.7: Repeatability scores of first egg date at different levels of year, individual and breeding colony, where age (actual) and latitude were considered as fixed effects.
Repeatability scores without covariates were also calculated

List of figures

Figure 2.1 A&B: 95% confidence interval from the model (LMM) estimate of the variables for fledge date for juvenile purple martins. The red bar shows estimate ± 1.96 *se, and the black bar color shows estimate \pm se (A). The correlation between first egg date and fledge date, gray Figure 2.2 A-D: 95% confidence interval from the model (LMM) estimate of the variables for colony departure date of juvenile (A) and adult purple martins (B). The red bar color shows an estimate ± 1.96 *se, and the black bar color shows an estimate \pm se (A & B). The correlation between first egg date and colony departure date of juveniles (C) and adults (D), gray shading Figure 2.3: The correlation between first egg date and age at colony departure date, grey Figure 2.4: Comparison of variation of fledge date and colony departure date among nestmates Figure 3.1: Duration in the nest for young in the treatment group that experienced extended day length as compared to young in the control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates mean. Whiskers extend to maximum and minimum values; outliers are indicated by Figure 3.2: Duration at the colony for young in the treatment group that experienced extended day length as compared to young in the control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates mean. Whiskers extend to maximum and minimum values; outliers are indicated by Figure 3.3: The influence of experimentally extended photoperiod on fledge and colony departure dates: a) shows the correlation between first egg date and fledge date; b) shows the correlation between first egg date and colony departure date. In figure 3 (a and b), each point Figure 4.1: The influence of ALAN (white and green lights) on fledge date and colony departure date, a) shows the correlation between first egg date and fledge date; b) shows the correlation between first egg date and colony departure date. Red= white light, Black: green Figure 4.2: Weights of young in the treatment groups (white and green lights) and control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates the mean. Whiskers extend to maximum and Figure 5.1: Fall stopover duration comparison between SY and ASY birds124 Figure 5.2: Spring stopover duration comparison between SY and ASY birds......124 Figure 5.3 A&B: Comparison of flight distance in fall (a) and spring (b) migrations between Figure 5.4 A-C: Age-related changes in average departure date (a), crossing tropic date (b) Figure 5.5 A_C: Age-related changes in average departure date (a), crossing tropic date (b)

Chapter 1

General introduction

Endogenous rhythm-generation in an organism, which was discovered in the latter part of the twentieth century, defines rhythm in the different time scales of circadian, circannual, circatidal, and circalunar (Sulzman et al. 1984, Daan 2010). The biological clock of an organism synchronizes the timing of this endogenous rhythm with environmental cues to generate an internal clock time of the organism at that particular moment (Helm et al., 2017).

Internal clock time helps an organism to keep track of time and to cue the timing of different physiological processes where there is not any clue of the time from the environment, such as during hibernation (Dunlap et al. 2004, Helm et al., 2017). Chronobiological studies include the investigation of the function of biological clocks and how an organism's internal clock is organized in relation to life-cycle events and responses to the environment (Schwartz et al., 2017). However, most research in this field has been conducted in a laboratory, and there is scant information about the function of biological clock time in natural ecosystems (Schwartz et al., 2017). On the other hand, ecological studies often neglect the biological clock in studies of the response of an organism to its environment (Daan 1982, Helm et al., 2017). Therefore, the new 'wild clock' approach provides an opportunity to understand timekeeping in free-living animals in natural ecosystems through integrating chronobiology and ecology (Helm et al., 2017).

To study animal timing in the natural ecosystem and investigate internal clock time in response to environmental cues or "*zeitgebers*" (Schwartz et al., 2017), using the 'wild clock' approach along with new animal-tracking technologies can provide new opportunities to study animal timing in natural ecosystems; which may be more complex and may differ from what has been found in laboratory studies (Vanin et al. 2012; Fuchikawa et al. 2016, Helm et al., 2017). *Zeitgebers* are external cues that modify an organism's behaviour and physiology

in response to biotic and abiotic conditions. These cues may be geophysical, light-based (photoperiod), social (mating opportunities), related to environmental conditions such as temperature, or food-based (availability) (Helm et al. 2017). Among *Zeitgebers*, the important role of the photoperiodic cycle (Aschoff 1955) has been discovered for endogenous timing programs or circannual cycles of an organism (Gwinner 1989, Berthold 1996). For example, previous laboratory studies have demonstrated the main role of photoperiod in timing of life-cycle events for birds, such as migration (Gwinner 1989, Gwinner, 1996). The evolution of endogenous timing programs over many years has regulated timing of circannual life-cycle events, such as migration, to align with appropriate environmental conditions (Gwinner, 1996). Knowledge of the way that photoperiod as a *Zeitgeber* can influence the timing of life-cycle events of a species, such as migration, in a natural environment can help to predict their responses to environmental change, such as with climate change or through the impact of anthropogenic light at night (Helm et al. 2017).

Previous studies have shown how the life-history events of migratory birds (mostly passerine) such as nesting experience (Coppack et al. 2001, Pulido and Widmer 2005, Mitchell et al. 2012), as well as environmental factors such as day length (Berthold 1996; Gwinner 1996; Dawson 2003), can interact with their heritable endogenous rhythms to influence fall migration timing, particularly in juveniles (Berthold 1996). Hence, the interaction of endogenous rhythm and experienced day length by nestlings, which is determined by their hatch date, could be influential on the timing of their subsequent life-cycle events (Berthold 1996, Hall and Fransson 2000). Despite the influence of weather conditions such as temperature and wind conditions on migration timing at finer, daily timescales (Åkesson & Hedenström 2000, Mitchell et al. 2012), the timing of nesting could determine the amount of time that a bird has before its migration. Therefore, a bird with a later hatch date that experiences a shorter day length, will have a smaller amount of time to

10

prepare for migration and complete the steps of moult and fat deposition (for migratory fuel) to avoid delays in the onset of migration. Laboratory studies have shown that post-juvenile moult and migratory behaviour of birds starts at a younger age when they experience shorter photoperiods (Berthold 1988, Berthold 1996, Coppack et al. 2001, Gwinner & Helm 2003). The development of these birds was also accelerated which has been described as a 'calendar effect' (Berthold 1988, Berthold 1993). The potential for earlier nesting to result in earlier migration timing through an ontogenetic effect of photoperiod (Both et al. 2010) suggests the possibility for phenotypic plasticity in migration timing of juvenile migratory birds through exposure to different environmental conditions or daylength in the nest. Phenotypic plasticity is the ability of an organism to change in morphology, physiology, or behaviour to respond to new environmental conditions (Price et al. 2003).

The advancement of spring phenology or the timing of life-cycle events, such as flowering and breeding in plant and animal species (Walther et al. 2002, Root et al. 2003, Dunn 2004), due to climate change (Visser et al. 2010, Thackeray et al. 2016) can also impact the timing of life-cycle events of migratory birds, such as breeding (Åkesson et al. 2017). Endogenous rhythms may have the main role in the timing of migration schedules of longdistance migrants (Gwinner 1996), as birds do not have information about the environmental conditions on their breeding grounds while at their distant overwintering sites (Both & Visser 2001). Photoperiod, which is the main cue in synchronizing endogenous systems with environmental conditions, governs the migration timing of long-distance migratory birds (Berthold 1996, Gwinner 1996, 1972). Therefore, any shift in breeding date could expose juveniles to a new photoperiod regime after hatching which could result in changes in their migration timing. In a laboratory experiment, the influence of simulating early spring photoperiod during the nesting phase resulted in an extension of the moulting phase and later fall migration (Coppack et al. 2001). Moreover, a delay in hatch date of nestlings through experimental manipulation of incubation in the wild resulted in a delay in spring arrival date the next year (Ouwehand et al. 2017). However, further studies are required to examine the impact of photoperiod on migration timing in free-living migratory songbirds (ontogenetic effect) based on the 'wild clock' approach.

Moreover, it is hypothesized that artificial light at night (ALAN) could be perceived as photoperiod (Farner 1964) which could be influential on endogenous timing systems (Berson et al. 2002) and consequently impact the timing of birds' activities such as singing (Kempenaers et al. 2010) and reproductive development (Dominoni et al. 2013) as well as physiological processes (Foster et al 2004) and natural life cycles (Rich and Longcore 2005). Additionally, the impact of ALAN on spring migration timing has been demonstrated, where birds who experienced ALAN for 10 nights at the wintering ground had later spring departure and arrival dates (Smith et al. 2021). Therefore, it could be hypothesized that ALAN is influential on migration timing and could impact the development of timing while birds are in the nest through an ontogenetic effect.

The endogenous timing of migratory birds may also be influenced by their ageing in response to various environmental, social, and physiological factors (Baker 1993, Hockey et al. 1998, Kokko 1999, McKinnon et al. 2014). During fall migration, juvenile songbirds mostly depend on their endogenous timing system (Berthold 1996, Gwinner 2003). During fall migration first-time migrants gain enough experience to modify their endogenous system and change their brains (Healy et al., 1996) which influences their ability to respond to varying environmental conditions and timing in the next spring migration (McKinnon et al., 2014). As previous studies have demonstrated, the delay of the fall migration of young migratory songbirds as compared to adults was not found in their subsequent spring migration (Schmaljohann et al. 2018).

Moreover, in raptors and shorebirds improvement in migratory strategies and route selection as birds age has been demonstrated (Hake et al. 2003, Lindström et al. 2011). These

12

differences in migratory traits between adults and young of a migratory bird could be explained by two proposed scenarios. The first one is based on young birds having less experience, making them less efficient in foraging (Mellone et al. 2013), flight (Alatalo 1984), and navigation (Crysler et al. 2016). The second scenario, based on Game-theory models, proposes that young birds have different optimal migration strategies in comparison with adults, leading young birds to employ strategies with the least costs. According to this scenario, younger birds may arrive later at breeding sites to avoid the negative impacts of competition with adults for defending territory and nests (when they are less likely to be successful), as well as the potential negative impacts of higher migratory speed and earlier arrival date at the breeding grounds when temperatures may be cool or variable and food limited. Instead, younger birds may not travel as quickly or choose longer routes which may help them to save more energy (McKinnon et al., 2014). Therefore, it is suggested that the endogenous system of young birds responds differently to photoperiod, or that other environmental cues influence the timing of their circannual activities (Berthold, 1996). Previous studies have mostly emphasized differences in migration strategies between young and adults based on the differences in their migration traits (e.g., Rappole 2013, McKinnon et al. 2014, Neufeld et al. 2021). However, the studies that investigate changes in migration traits as birds age have rarely examined migration behaviour over more than one year or full migration routes (e.g., Sergio et al. 2014, Fayat 2020).

Purple martin as a study system

Purple martins (*Progne subis*) are a gregarious, long-distance, Nearctic-Neotropical migratory songbird species that journeys 10-20,000 km between breeding sites across North America and overwintering locations in South America (Fraser et al., 2012). This species is one of the aerial insectivorous (birds that consume insects while flying) migrants that have suffered from population declines since 1970, mostly in the northern part of their breeding range (Nebel et al. 2011; Smith et al. 2015; Michel et al. 2016). During the last two decades, their populations in the Southern and Gulf Coast states have also been experiencing a decline (Tautin et al. 2009; Ray

2015; Sauer et al. 2017). This species has been selected for this study because of their wide distribution, local abundance, accessibility of breeding colonies, and an existing database of migration timing for individuals collected through field studies. Moreover, there is evidence that migratory behaviour of martins may not respond to climate change-driven temperature anomalies (Fraser et al. 2013). However, Shave et al. (2019) showed population-level change in egg laying date of purple martins in response to changes of temperature suggesting the potential for individual plasticity to temperatures once birds are at the breeding ground. Therefore, further investigation, including experimental approaches, are required to further investigate the phenotypic plasticity of timing to environmental factors.

Objectives:

Previously, chronobiologists studied biological clock time using laboratory studies only, thus their understanding of the function and evolution of internal clock time was limited. Meanwhile, timekeeping systems were undefined in ecological studies, despite their focus on understanding relationships between the behaviour of an organism and its environment (Helm et al. 2017). Therefore, the new 'wild clock' approach is beneficial to advancing our understanding of timekeeping systems in animals because it places biological clock functions familiar to chronobiologists in the context of complicated environmental factors in the wild (Vanin et al. 2012, van der Vinne et al. 2014, Fuchikawa et al. 2017, Helm et al. 2017). New powerful animal tracking technologies can be applied in studies using the 'wild clock' approach to gather timing and movement data in a wild environment (Nord et al .2016, Helm et al. 2017). Therefore, the main objective of this thesis is to investigate factors that influence the development and maintenance of migration timing of a long-distance migratory bird by using the 'wild clock' approach and new tracking technologies.

I wrote this thesis in manuscript form and it contains four data chapters addressing nest timing and post-breeding movement timing, photoperiod and post-breeding movements timing, anthropogenic light at night (ALAN) and post-breeding movement timing, and ageing and

14

migratory traits and timing in the purple martins. First, I studied whether nest timing (first egg date) and environmental factors predict the timing of post-breeding movements of juveniles and adults (Chapter 2; In press Journal of Avian Biology, DOI: <u>https://doi.org/10.1111/jav.02766</u>). Next, I used an experimental approach where I manipulated photoperiod during nestling development and investigated the subsequent impacts on the timing of post-breeding movements in the wild (Chapter 3; published Proceedings of the Royal Society b. doi: <u>https://doi.org/10.1098/rspb.2021.1474</u>). I also examined the impact of ALAN during nestling development to determine the potential impact on timing of post-breeding movements (Chapter 4; published Frontiers in Ecology and Evolution, <u>https://doi.org/10.3389/fevo.2021.735112</u>). In my final data chapter, I studied the migration strategies across age groups and to determine how migration strategies may differ as birds age (Chapter 5). Collectively these chapters address the knowledge gaps in our understanding of migration timing development and its function in long-distance migratory birds by using the 'wild clock' approach.

References:

- Åkesson, S., and Hedenström, A. 2000. Wind selectivity of migratory flight departures in birds. BEHAV ECOL SOCIOBIOL. 47(3): 140-144.
- Åkesson, S., Ilieva, M., Karagicheva, J., Rakhimberdiev, E., Tomotani, B., & Helm, B. (2017). Timing avian long-distance migration: from internal clock mechanisms to global flights. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1734), 20160252.
- Alatalo, R. V., Gustafsson, L., & Lundbkrg, A. (1984). Why do young passerine birds have shorter wings than older birds?. *Ibis*, *126*(3), 410-415.
- Aschoff, J. (1955). Jahresperiodik der Fortpflanzung bei Warmblütern. Studium generale, 8(12), 742-776.
- Baker, R. R. (1993). The function of post-fledging exploration: a pilot study of three species of passerines ringed in Britain. *Ornis Scandinavica*, 71-79.
- Berson, D. M., Dunn, F. A., & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science*, 295(5557), 1070-1073.
- Berthold, P. 1993. Bird migration: a general survey. Oxford, Oxford University Press
- Berthold, P. (1996). Control of bird migration. Springer Science & Business Media.
- Berthold, P. (1988). The control of migration in European warblers. In XIX Congressus Internationalis Ornithologici (pp. 215-249). University of Ottawa Press.
- Both, C., Van Turnhout, C. A., Bijlsma, R. G., Siepel, H., Van Strien, A. J., & Foppen, R. P. (2010). Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society B: Biological Sciences*, 277(1685), 1259-1266.
- Both, C., & Visser, M. E. (2001). Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature*, *411*(6835)
- Coppack, T., Pulido, F. and Berthold, P. 2001. Photoperiodic response to early hatching in a migratory bird species. Oecologia. 128(2): 181-186.
- Crysler, Z. J., Ronconi, R. A., & Taylor, P. D. (2016). Differential fall migratory routes of adult and juvenile Ipswich Sparrows (Passerculus sandwichensis princeps). *Movement ecology*, 4
- Daan, S. (1982). Circadian contributions to survival. [In: Vertebrate circadian systems. J. Aschoff, S. Daan and GA Groos, eds].
- Daan, S. (2010). A history of chronobiological concepts. In *The circadian clock* (pp. 1-35). Springer, New York, NY.
- Dawson, A. (2003). Photoperiodic control of the annual cycle in birds and comparison with mammals. *Ardea*, *90*(3), 355-367.
- Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society B: Biological Sciences*, 280(1756), 20123017.
- Dunlap, J. C., Loros, J. J., & DeCoursey, P. J. (2004). *Chronobiology: biological timekeeping*. Sinauer Associates.
- Dunn, P. (2004). Breeding dates and reproductive performance. Advances in ecological research, 35, 69-87.
- Farner, D. S. (1964). The photoperiodic control of reproductive cycles in birds. *American Scientist*, 52(1), 137-156.
- Fayet, A. L. (2020). Exploration and refinement of migratory routes in long-lived birds

- Foster, R., Kreitrman, L., & Roenneberg, T. (2004). Books and arts-Rhythms of Life: The Biological Clocks that Control the Daily Lives of Every Living Thing. *Nature*, 427(6977), 784-784.
- Fraser, K. C., Silverio, C., Kramer, P., Mickle, N., Aeppli, R., & Stutchbury, B. J. (2013). A trans-hemispheric migratory songbird does not advance spring schedules or increase migration rate in response to recordsetting temperatures at breeding sites. *PLoS One*, 8(5), e64587.
- Fraser, K. C., Stutchbury, B. J., Silverio, C., Kramer, P. M., Barrow, J., Newstead, D., ... & Tautin, J. (2012). Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. *Proceedings of the Royal Society B: Biological Sciences*, 279(1749), 4901-4906.
- Fuchikawa, T., Beer, K., Linke-Winnebeck, C., Ben-David, R., Kotowoy, A., Tsang, V. W. K., ... & Bloch, G. (2017). Neuronal circadian clock protein oscillations are similar in behaviourally rhythmic forager honeybees and in arrhythmic nurses. *Open biology*, 7(6), 170047.
- Fuchikawa, T., Eban-Rothschild, A., Nagari, M., Shemesh, Y., & Bloch, G. (2016). Potent social synchronization can override photic entrainment of circadian rhythms. *Nature communications*, 7(1), 1-10.
- Gwinner, E. (1972). Adaptive functions of circannual rhythms in warblers. In XVth Congressus Internationalis Ornithologici (pp. 218-236). EJ Brill.
- Gwinner, E. (1996). Circadian and circannual programmes in avian migration. The Journal of Experimental Biology, 199(1), 39-48.
- Gwinner, E. (1989). Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. *Journal of Biological Rhythms*, 4(2), 125-138.
- Gwinner, E., & Helm, B. (2003). Circannual and circadian contributions to the timing of avian migration. In Avian migration (pp. 81-95). Springer, Berlin, Heidelberg.
- Hake, M., Kjellén, N., & Alerstam, T. (2003). Age-dependent migration strategy in honey buzzards Pernis apivorus tracked by satellite. *Oikos*, *103*(2), 385-396.
- Hall, K. S. S. and Fransson, T. 2000. Lesser Whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. J AVIAN BIOL. 31(4): 583-587.
- Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1734), 20160246.
- Healy, S. D., Gwinner, E., & Krebs, J. R. (1996). Hippocampal volume in migratory and non-migratory warblers: effects of age and experience. *Behavioural brain research*, *81*(1-2), 61-68.
- Hockey, P. A., Turpie, J. K., & Velásquez, C. R. (1998). What selective pressures have driven the evolution of deferred northward migration by juvenile waders?. *Journal of Avian Biology*, 325-330.
- Kempenaers, B., Borgström, P., Loës, P., Schlicht, E., & Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Current Biology*, 20(19), 1735-1739.
- Kokko, H. (1999). Competition for early arrival in migratory birds. Journal of Animal Ecology, 68(5), 940-950.
- Lindström, Å., Gill Jr, R. E., Jamieson, S. E., McCaffery, B., Wennerberg, L., Wikelski, M., & Klaassen, M. (2011). A puzzling migratory detour: are fueling conditions in Alaska driving the movement of juvenile Sharp-tailed Sandpipers?. *The Condor*, 113(1), 129-139.
- McKinnon, E. A., Fraser, K. C., Stanley, C. Q., & Stutchbury, B. J. (2014). Tracking from the tropics reveals behaviour of juvenile songbirds on their first spring migration. *PloS one*, *9*(8), e105605.

- Mellone, U., López-López, P., Limiñana, R., Piasevoli, G., & Urios, V. (2013). The trans-equatorial loop migration system of Eleonora's falcon: differences in migration patterns between age classes, regions and seasons. *Journal of Avian Biology*, 44(5), 417-426.
- Mitchell, G. W., Newman, A. E., Wikelski, M. and Ryan Norris, D. 2012. Timing of breeding carries over to influence migratory departure in a songbird: an automated radiotracking study. J ANIM ECOL. 81(5): 1024-1033.
- Michel, N. L., Smith, A. C., Clark, R. G., Morrissey, C. A., & Hobson, K. A. (2016). Differences in spatial synchrony and interspecific concordance inform guild-level population trends for aerial insectivorous birds. *Ecography*, 39(8), 774-786.
- Nebel, S., Mills, A., McCracken, J., & Taylor, P. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology*, 5(2).
- Neufeld, L. R., Muthukumarana, S., Fischer, J. D., Ray, J. D., Siegrist, J., & Fraser, K. C. (2021). Breeding latitude is associated with the timing of nesting and migration around the annual calendar among Purple Martin (Progne subis) populations. *Journal of Ornithology*, 1-16.
- Nord, A., Lehmann, M., MacLeod, R., McCafferty, D. J., Nager, R. G., Nilsson, J. Å., & Helm, B. (2016). Evaluation of two methods for minimally invasive peripheral body temperature measurement in birds. *Journal of Avian Biology*, 47(3), 417-427.
- Ouwehand, J., Burger, C., & Both, C. (2017). Shifts in hatch dates do not provide pied flycatchers with a rapid ontogenetic route to adjust offspring time schedules to climate change. *Functional Ecology*, *31*(11), 2087-2097.
- Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1523), 1433-1440.
- Pulido, F. and Widmer, M. 2005. Are Long-Distance Migrants Constrained in Their Evolutionary Response to Environmental Change? Causes of Variation in the Timing of Autumn Migration in a Blackcap (S. atricapilla) and Two Garden Warbler (Sylvia borin) Populations. ANN NY ACAD SCI. 1046(1): 228-241
- Rappole, J. (2013). The avian migrant. Columbia University Press.
- Ray, J. D. (2015). The status of the Purple Martin in Texas. Bulletin of the Texas Ornithological Society, 48(1-2), 41-46.
- Rich, C., and Longcore, T. (2005). Ecological Consequences of Artificial Night Lighting. Washington, DC: Island Press.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., & Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*
- Sauer, J. R., Pardieck, K. L., Ziolkowski Jr, D. J., Smith, A. C., Hudson, M. A. R., Rodriguez, V., ... & Link, W. A. (2017). The first 50 years of the North American breeding bird survey. *The Condor: Ornithological Applications*, 119(3), 576-593.
- Sergio, F., Tanferna, A., De Stephanis, R., Jiménez, L. L., Blas, J., Tavecchia, G., ... & Hiraldo, F. (2014). Individual improvements and selective mortality shape lifelong migratory performance. *Nature*, 515(7527), 410-413.

Schwartz, W. J., Helm, B., & Gerkema, M. P. (2017). Wild clocks: preface and glossary.

- Schmaljohann, H., Mueller, F., Klinner, T., & Eikenaar, C. (2018). Potential age differences in the migratory behaviour of a nocturnal songbird migrant during autumn and spring. *Journal of Avian Biology*, 49(7), e01815.
- Shave, A., Garroway, C. J., Siegrist, J., & Fraser, K. C. (2019). Timing to temperature: egg-laying dates respond to temperature and are under stronger selection at northern latitudes. *Ecosphere*, *10*(12), e02974.
- Smith, R. A., Gagné, M., & Fraser, K. C. (2021). Pre-migration artificial light at night advances the spring migration timing of a trans-hemispheric migratory songbird. *Environmental Pollution*, 269, 116136.
- Smith, A. C., Hudson, M. A. R., Downes, C. M., & Francis, C. M. (2015). Change points in the population trends of aerial-insectivorous birds in North America: synchronized in time across species and regions. *PloS one*, 10(7), e0130768.
- Sulzman, F. M., Ellman, D., Fuller, C. A., Moore-Ede, M. C., & Wassmer, G. (1984). Neurospora circadian rhythms in space: a reexamination of the endogenous-exogenous question. Science, 225(4658), 232-234.
- Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe, S., ... & Wanless, S. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, 535(7611), 241-245.
- Tautin, J., Cousens, B., Kostka, K., Kostka, S., Airola, D. A., Rich, T., ... & Thompson, C. (2008, February). Addressing regional declines in Purple Martin populations. In *Tundra to tropics: Connecting birds, habitats and people. Proceedings of the 4th International Partners in Flight Conference* (pp. 13-16).
- van der Vinne, V., Riede, S. J., Gorter, J. A., Eijer, W. G., Sellix, M. T., Menaker, M., ... & Hut, R. A. (2014). Cold and hunger induce diurnality in a nocturnal mammal. *Proceedings of the National Academy of Sciences*, 111(42), 15256-15260.
- Vanin, S., Bhutani, S., Montelli, S., Menegazzi, P., Green, E. W., Pegoraro, M., ... & Kyriacou, C. P. (2012). Unexpected features of Drosophila circadian behavioural rhythms under natural conditions. *Nature*, 484(7394), 371-375.
- Visser, M. E., Caro, S. P., Van Oers, K., Schaper, S. V., & Helm, B. (2010). Phenology, seasonal timing and circannual rhythms: towards a unified framework. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1555), 3113-3127.
- Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., ... & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, 416(6879), 389-395.

Chapter 2: Does hatch date set the clock? Timing of post-fledging movements for families of a colonially breeding, long-distance migratory songbird

Saeedeh Bani Assadi¹, Emily A. McKinnon², Edward D. Cheskey³, Kevin C. Fraser¹

¹Department of Biological Science, University of Manitoba

² Access & Aboriginal Focus Programs, University of Manitoba

³ Nature Canada, 300-240 Bank Street, Ottawa, Ontario K2P 1X4

Status; In press Journal of Avian Biology (DOI: https://doi.org/10.1111/jav.02766)

Abstract

Factors that influence the development of migration timing in juvenile songbirds have implications for the ability of individuals to respond positively to rapid environmental changes. We investigated the impacts of nest timing on the post-fledging movement timing of juveniles and adults of a migratory songbird. We tested whether first egg date and environmental factors predicted the initiation of post-fledging stages: fledge date for juveniles, and colony departure date for both adults and juveniles. At breeding colonies of purple martin (Progne subis) in southern Ontario, Canada, we monitored nests to determine the date of nest initiation ('first egg date') and deployed 122 coded radio-frequency tracking tags on young and adults to determine the timing of post-fledging stages. We found that first egg date, the number of nestmates and age of parents were the main predictors of fledge date. Of these three factors, only first egg date carried through post-fledge to influence colony departure date for juvenile birds, but this relationship weakened between first egg date and departure date. Nestmates tended to fledge together (range 0-4 days) but exhibited greater variation in colony departure timing (range 0-11 days). Further, while first egg influenced departure date, increasing variation between fledge and departure led to some birds departing breeding colonies at a younger age, suggesting in influence of local environmental factors (e.g. social or photoperiod change) in departure decisions. The timing of adult colony departure date was independent of first egg dates. In sum, our results suggest a role for first egg dates in setting timing of postbreeding movements, but with variation introduced between fledge and departure dates. Experimental manipulations of photoperiod in a wild setting are needed and future research should investigate whether the timing of movement driven by nesting timing, holds across the rest of migration or even the lifetime of birds.

Keywords: automated telemetry, endogenous timing, internal clock time, juvenile migration, post-fledging, calendar effect, purple martin, Progne subis, aerial insectivore

21

Introduction:

Endogenous mechanisms in combination with information from exogenous factors, shape "internal clock time" of migratory songbirds which provides cues for the timing of life history events (Berthold 1996, Gwinner 1996a, Helm et al. 2017). An organism synchronizes the timing of its activities by the response of its biological clock to information from environmental factors (Helm et al. 2017). Previous studies have shown the important role of heritable endogenous mechanisms in the migration timing of juvenile (mostly passerine) birds (Berthold 1996, Gwinner 1996b, Pulido et al. 2001, Gwinner 2003, Both 2010), which can be modified by life-history events such as the timing of breeding and the natal circumstances experienced by young (Pulido et al. 2001, Coppack et al. 2001, Pulido and Widmer 2005, Mitchell et al. 2012). Hatch date determines the day-length experienced by a nestling which is predicted to interact with endogenous mechanisms to cue subsequent migration timing (Berthold 1996, Hall and Fransson 2000). Nest timing can also constrain the amount of time available for a bird to prepare for migration. Later hatched birds may prepare for migration in a shorter period of time, which has been described as a 'calendar effect' (Berthold 1988, Berthold 1993). On a smaller timescale (days or within a day), migration timing might be determined by the availability of favourable weather conditions (Åkesson and Hedenström 2000, Mitchell et al. 2012).

Improving our understanding of the relative plasticity of migration timing to environmental stimuli could provide invaluable insight into the flexibility of migratory behaviour to rapid environmental change (Knudsen et al. 2011). Despite the important role of endogenous mechanisms in migration timing demonstrated through lab studies (e.g., Gwinner 2003, Gwinner 1996b), we need to know about their role in the wild, such as their potential impact on plasticity. For example, it has been proposed that earlier nesting results in advanced migration timing of juveniles through an ontogenetic effect of photoperiod in the nest (Both 2010). This was not corroborated by a follow-up experimental study (Ouwehand et al. 2017), but this requires further investigation in other systems. Fledge date may also limit the time available to prepare for fall migration with potential carry-over effects of late nesting on survival (Grüebler and Naef-Daenzer 2010, Tarof et al. 2011, Cox et al. 2014). However, the post-breeding period of young and adult long-distance migratory songbirds is still relatively under-studied (Kershner et al. 2004, Vitz and Rodewald 2011, Naef-Daenzer and Gruebler 2016), largely due to difficulties in tracking post-breeding movements (De Frutos and Olea 2008). New automated, radio-telemetry systems such as the Motus Wildlife Tracking System provide the opportunity to investigate specific timing events within the post-breeding periods of adult and juvenile birds (Taylor et al. 2017) when mortality rate of juveniles is high. Also, retrieval of other types of direct-tracking archival tags, such as geolocators or GPS units, would be logistically difficult.

Here, we investigate the influence of multiple biotic and abiotic factors on the timing of post-breeding stages of juvenile, long-distance migratory songbirds (purple martins *Progne subis*): 1) fledge date and, 2) breeding colony departure date, reflecting the start of the first step of broader post-breeding movements. By mid-August to late September in Canada, purple martins, which are aerial insectivores, start fall migration to their South American overwintering grounds, which are mostly in the Amazon basin of Brazil (Sick 1993, Fraser et al. 2017). We explored multiple factors that may influence the timing of post-breeding stages including nest timing (first egg date), the number of nestmates (brood size), age of parents (second year or after second year, henceforth 'SY' and 'ASY', respectively), and weight and fat scores of juveniles. According to the "kin selection hypothesis", which suggests nestmates wait for each other to reach a good body condition for fledging (Freed 1988), we predicted fledge date would be more similar among nestmates (assumed to be full siblings of a social pair) than those of other cavities at the same colony (assumed to be non-siblings). Multiple

factors may influence nestling weight, but since purple martin nestlings can lose weight to achieve the appropriate weight for flight as they approach fledge date (Allen and Nice 1952, Dellinger and Rogillio 1991, Gagné 2019), we predicted that juveniles that were a lighter weight at the time of tagging (near fledge), would fledge earlier (Allen and Nice 1952, Dellinger and Rogillio 1991, Gagné 2019). If the timing of a nest has a broad, overall impact on the timing of colony departure, then we also predicted that departure date would be more similar for parents and their chicks (broods) as compared to adults and juveniles from other cavities. We also investigated the influence of first egg date, the number of nestmates, and parents' age on colony departure date of adults. Lastly, we tested the 'calendar effect' hypothesis, where we predicted that later nest timing leads to a shorter time at the breeding colony and relatively earlier initiation of post-breeding movement at a younger age for juveniles.

Methods:

This study was conducted at two different breeding colonies of purple martin in southern Ontario, Canada, during July 2017 and 2018 and at an additional breeding colony in July 2019. Colony sites were along Lake Erie and Lake Ontario at Holiday Beach Conservation area (42.05°, -83.04°) and at a private residence at Port Bruce (42.66°, -81.06°) during the first and second year, and at Ruthven park (42.97°, -79.87°) in the third year of the study. These colonies were strategically selected as they were in the middle of the densest array of Motus receivers and their distances to the closest motus tower are in a range of 0.04-0.8 km, (see <u>www.motus.org</u>) which was predicted to provide more complete data on the post-breeding movements of martins than could be accomplished at other locations.

We captured birds by using drop-door traps at their breeding nest boxes. To track individuals, we used the Motus Wildlife Tracking System (<u>www.motus.org</u>). This system is an international, automated radio-telemetry array of receiving stations (Taylor et al. 2017) that

can be used to track the movement of small animals, and is thus ideal for tracking songbirds such as purple martins and their movements soon after fledging. Signals were emitted every 10 seconds (bursts) from individually coded radio frequency NTQB-3-2 Avian NanoTags (0.67 g; 124 days expected tag life; Lotek Wireless Inc., on the same frequency 166.380 MHz) which were mounted on individual birds and were detected by the receiver stations in the Motus network. The tags were mounted on adults and juvenile birds which were near fledging by using a figure-eight leg-loop harness (Rappole and Tipton 1991). We mounted tags on a total of 122 purple martins; with 55 NanoTags deployed during the first 2 years, and 12 deployed in the third year (13 adult males, 21 adult females, and 88 fledglings). The average weight of adult purple martin is 51.4 ± 4.54 grams (63 measured adults) and the average weight of young near fledging is 50 grams (Allen and Nice 1952, Dellinger and Rogillio 1991), so the weight of the tags (0.75 grams with harness plus the metal band weight at 0.16 grams) was not more than 3% of their total body weight. Adult birds were aged and sexed by using plumage characteristics (Pyle 1997). Nestling birds could not be sexed visually.

Tag detections by Motus antenna were recorded by SensorGnomes at each Motus receiving station (Taylor et al. 2017). Following established protocols, all data were post-processed to identify the registered tag in the project, tower location, antenna direction, signal strength, time, and to identify and remove false-positive detections (Crewe et al. 2018). We considered a minimum of 4 consecutive tag bursts (run length = 4) detected at a receiver as a valid detection (Baldwin et al. 2018). Also, any detection at a location that was too far away from the last approved detection, such that travel speeds between the points would be unrealistic (average migration rate of purple martin is ~6 m/s, unpublished data based on GPS tracking of purple martins), were removed. Birds were tagged near their fledge date which is typically between 27 to 36 days old (Allen and Nice 1952). At the time of tagging, the nestling age was between 17 and 25 days old. Their weight (to the nearest 0.1g) and fat content (scored

from 0-5, following MoSI protocol, De Sante et al. 2009) were measured. We used weight and fat measurements in our fledge date model, but we did not include these variables in our investigation of colony departure date, because it was not possible to re-capture juvenile birds once they had fledged to obtain weight and fat score relevant to departure timing.

We determined fledge date, considering the age of juveniles and tag signal strength fluctuations measured by the receiver. The first time the signal strength of a tagged young dramatically increased or decreased after a long period of stability (when birds were stationary in their nest cavity before fledging), indicating the individual had left the cavity. Further fluctuations of signal strength afterwards provided further indication that birds had fledged and were moving closer or further to the antenna during first flights (Supplemental Fig. 2.1). The colony departure date was determined when there were no further detections of the bird at the colony site after fledging for more than one day and in some cases were further supported by detections from other receivers in the Ontario array away from the colony site (Gómez et al. 2017) (Supplemental Fig. 2.1). We found that the accuracy of this method for determining the fledge date was ~96%, based on tests in other colonies of purple martin in Manitoba where fledge dates were confirmed by daily nest checks and observations of banded birds at colonies (fledge dates of 31 out of 32 nests based on Motus signals matched with our field observations, with the one mismatch being where fledge date based on Motus detections was a range of July 24-26, where our field observations in this case could only confirm July 24) (Bani Assadi and Fraser 2021).

This work was performed under the University of Manitoba's Animal Care Committee protocol F18-031/1 (AC11388).

Statistical Analysis:

To determine the influence of nest timing (first egg date) and environmental factors on fledge date and initiation of broader post-breeding movements, we used linear mixed models

26

(LMM) and linear models (LM) using the R package *lme4* (Bates et al. 2014). Global models for the two timing stages included year, colony site, and cavity as random effects and fixed effects of first egg date, number of nestmates, age of parents ('SY' and 'ASY'). Where the age of both parents was known, pairs were the same age (ASY:ASY or SY:SY), but for nests where the age of only one parent was known, we cannot rule out the potential for mixed-age pairs. However, most birds may mate assortatively (Morton and Derrison 1990), where in 2020 in a Manitoba colony the majority of pairings were ASY: ASY (65%) with a smaller percent being SY:SY (4.25%) (Fraser unpublished data) therefore we anticipate little impact of potential mixed-age pairs on our results. The global model for the first fledge date for juveniles also included fat score (from 0 to 5) and weight (g). No collinearity was found among fixed factors of the models. The global models of departure date from a colony were run for adults and juveniles, separately. We also used two linear regression models (LM) to investigate the relationship between first egg date, number of nestmates and age of parents with the age of juveniles at fledge date and colony departure date. The residuals were distributed normally for our models except the model for age at fledge date model which we. ran by using Generalized Linear Models (glm). The residuals of the juvenile colony departure date model had positive skewness and it did not fit with other modelling approaches (e.g. Poisson regression, transformations), and so the linear mixed effect model was run (Deakin et al. 2019). We used AICc (Akaike's Information Criterion corrected for small sample size) (MuMIn R package Barton 2013) scores for ranking all models according to parsimony (supplementary table 2.1-2.3). For model selection, we used to $\Delta AICc < 2$ to select candidate models with the biological covariates that were most influential on the response variable (Burnham and Anderson 2002). The Akaike weight (w) was calculated (MuMIn R package Barton 2013) for each candidate model and used for the selection of the best model (Burnham and Anderson 2002). Wilcoxon

rank-sum tests were used to compare the timing of colony departure date of adults and juveniles. All analyses were conducted in R version 3.6.1. (R Core Team 2019).

Initial examination of the models revealed that the model of fledge date and colony departure date only converged with the random effect of cavity. The effect of year and site was tested by likelihood ratio tests comparing the models including each of these random effects with the models including cavity as a random effect. The results of these models were insignificant ($\chi^2 = 0$, P=1, for both variables and three models). Therefore, the random effects, 'year' and 'site' were removed from the models for parsimony.

Results:

We tracked the nest timing and post-breeding movements of a total of 88 juveniles and 34 adults (25 nests). During the post-breeding period, we detected 88% (30/34) of tagged adults and 85% (75/88) of tagged juveniles. Detections that were limited to nests before fledge date and random false positive detections were omitted. For most individuals, detections were limited to the colony site (83%). In instances where tags fell off at the breeding site (indicated by a steady, non-varying signal strength with a non-moving tag) or where there was no detection of the tag just after fledging, associated data were removed from colony departure date analysis. Our resulting sample size was 62 juveniles for fledge date, and 21 adults (2 SY and 6 ASY males; 6 SY and 7 ASY females) and 44 juveniles for departure date from the colony.

Fledge date

Fledge dates were 8-24 July during the three years of the study. Nestmate's fledge dates varied by 0-4 days. Later first egg date and having at least one younger (SY) parent resulted in later fledge dates by 1.03 ± 0.08 days (95% CI 0.87 to 1.20) and 1.79 ± 0.81 days (95% CI 0.19 to 3.38), respectively (Fig. 1A-B). Birds with more nestmates fledged later, whereby increasing the number of nestmates by one resulted in fledge dates that were 1.13 ± 0.36 days later (95%

CI 0.42 to 1.83) (Fig. 1A). The best fledge date model did not include weight or fat as influential factors (Table 1). We also did not find a relationship between age of juveniles at fledge date or age of parents on fledge date.

Colony departure date

Colony departure dates of nestlings were between July 14^{th} to August 5^{th} , and colony departure dates of adults were between July 13^{th} to August 7^{th} . Colony departure date varied between 0 to 11 days (3.66 ± 1.13) among nestmates of 11 cavities that had colony departure date information from at least two nestlings. Where we had data for both adults at a nest (3 pair), departure date varied by 1-7 days (3.33 ± 1.51) within pairs (Supplemental figure 2.4A).

The most important predictor of colony departure dates for juveniles was their first egg date. Juveniles departed the colony 0.52 ± 0.26 days (95% CI 0.00 to 1.04) later for every day that first egg date was later (Fig. 2A & C). Despite this, juveniles with later first egg dates departed the colony at a younger age (-0.46 ± 0.21, 95%, CI -0.88 to -0.03) (Fig. 3), (Supplemental Figure 2.3). None of first egg date, age of adults, or number of nestlings were influential on the colony departure date of adults (Fig. 2B & D) (Table 1).

Overall, juveniles did not depart the colony significantly later than adults. On average juveniles and adults departed the colony on July 23^{rd} (204.06 ± 0.80) and 22^{nd} (203.79 ± 0.82), respectively (W = 532, P = 0.32) (Supplemental figure 2.4B).

Discussion:

Our results demonstrate that the timing of fledge and departure from the natal colony are influenced by the timing of nest initiation and that juveniles from later nests depart the colony at a younger age. However, variation in timing increased between fledge date and colony departure date, suggesting the potential for an intervening influence of local environmental and social factors on the timing of juvenile bird departure from their colonies for the first time.

Our finding that the timing of nesting influences the timing of subsequent post-breeding movements in a wild population of birds aligns with laboratory-based studies of songbirds. These studies had demonstrated a strong connection between photoperiod experienced after hatch date and the timing of seasonal behaviours (e.g. Gwinner 1989, 1996b). For example, a recent study using pied flycatchers (Ficedula hypoleuca) showed a connection between nest timing and the timing of juvenile post-breeding behaviour, where hatch date was positively correlated with the timing of autumn moult (Helm et al. 2019), which was also supported by results in earlier lab studies (e.g. Gwinner 1986, 1989, Berthold 1988, Noskov et al. 1999). Early-hatched birds that experienced longer photoperiod, had earlier fledge dates and consequently began fall migration sooner (Coppack and Pulido, 2004). Field-based evidence is rarer, but a few studies using mark-recapture methods have demonstrated an influence of nest timing on subsequent post-breeding timing events (Ellegren 1990, Morton and Pereyra 1994, Sokolov 2000). A study that used similar (to our study) automated radio-telemetry methods also demonstrate the influence of hatch date and weather on precise estimates of departure timing in savannah sparrows (Mitchell et al. 2012). Our study builds upon these previous lab and limited field-based research. Our examination of the influence of nest timing across two stages of post-breeding movements in a colonially breeding, gregarious songbird also shows an influence of nest timing on both fledge and colony departure dates. Environmental factors associated with group living may serve to dilute the influence of nest timing on movements between fledge date and colony departure, but this requires further investigation. In a parallel investigation, we manipulated photoperiod in the wild and found that fledge and departure dates of young purple martin were impacted by photoperiod experienced in the nest. (Bani Assadi and Fraser 2021). These results suggest a strong role for photoperiod in timing that is independent of social or environmental cues, as birds fledge the nest and begin their broader post-breeding movements for the first time.

While we found earlier nesting resulted in earlier departure date of juveniles, our results also reveal that birds from nests with later first egg dates departed the colony at a younger age. This shorter interval between fledge date and colony departure date for later nests could be explained by social factors, predation pressure, or possibly seasonal changes in photoperiod cues ("calendar effect"). For the "calendar effect", juveniles with later fledge dates may be induced through photoperiod cues to start moulting at a younger age, thus compensating for their later fledge date and avoiding delays in migration (Jenni and Winkler 1994, Berthold 1993, Noskov et al. 1999, Bojarinova et al. 2008, 2010, Newton 2011). Our results also align with Brown's observational evidence (1978) that showed that for purple martins breeding at the southern extent of their range in Texas, juveniles that fledged before 15 June, stayed longer at the colony than those fledged later than that date (Brown 1978). Also consistent with our results, a lab-based study of Helm and Gwinner (2006) showed that later-hatched juvenile stonechats (Saxicola torquata) advanced their Zugunruhe to start migration activity roughly at the same time as earlier hatched individuals. One speculation regarding the calendar effect in purple martins is that exposure to natural photoperiod variation after fledging (when daylengths are getting shorter more rapidly) induces birds from later nests to start broader post-breeding movements earlier, enabling them to depart their colony at a similar time to birds from earlier nests. However, it is unknown whether this mechanism is an inherited endogenous response to the time of the year (according to photoperiod) or is related to food availability (Newton 2010), precipitation, or other seasonal variables like temperature (Hazra et al. 2012). Photoperiod might be considered as one of the main factors driving a potential calendar effect, as it would be a more consistent and reliable cue by latitude (Hazra et al. 2012) and it has an important role in regulating internal clock time (Gwinner, 2003). We can confirm that later hatching birds departed the colony at a younger age and that it is plasticity, not a lower survival rate among later hatched birds that contributed to this pattern because variation in signal strength and directional movement at departure noted by the motus receiver antenna indicate these were true departures while the tags were carried by living birds.

In support of social influences on the timing of post-breeding movements, we found that there was no significant difference between the colony departure date of adults and juveniles. There is some observational evidence that juveniles follow adults to communal grouping areas away from the colony, where they may benefit from food provisioning by adults and defense against predators (Brown 1978). These or other social factors could be a reason for later fledged juveniles joining their colony members, even if this means departing the natal colony at a younger age than birds from earlier nests.

Predation pressure may be a further factor that promotes more synchronous colony departure. There is no supporting evidence that predation pressure at colonies may promote earlier departure by later fledging birds, but we might expect that the threat of predation would be higher with fewer birds present to engage in communal defense strategies. How the threat of predation may promote more synchronous colony departure for early and late fledging juveniles would be a valuable avenue for future research.

The timing of fledge was largely synchronous within a cavity (with > 3 young), with most individuals fledging at the same date or within 1-4 days of each other (Supplemental Figure 2.2) which may be typical of within-nest variation in purple martins (Brown 1978). The later fledge dates with increasing brood size that we observed might be most simply explained by a later start to incubation for nests with larger broods. This would be where larger clutches are completed later, leading to later incubation and a subsequently later fledge date. However, alternative explanations may include the kin selection hypothesis (Freed 1988), which predicts the fledging of more developed nestlings is postponed until nestmates are more synchronized, to optimize fitness by increasing the chances of success of siblings with shared genes. Some evidence to support this hypothesis was provided through a study of 84 species of North American songbirds (Remeš 2007). A complementary or alternative explanation for our result is that increasing food competition in larger broods leads to delays in reaching optimal body conditions for fledging (Wagner et al. 1996). However, in this study weight or fat score were not influential factors on fledge date, which does not support the notion that later fledge dates may be explained by food competition. Variation in our sampling of weight (at days 17-26) overlaps with when purple martins reach their peak nestling weight at 17-21 days old (Brown and Tarof 2020), however some variation may have been introduced over this period that may have influenced our results regarding a weight effect. Lastly, space limitation within nest cavities could delay fledge dates, where larger broods are more confined limiting flight muscle exercise and consequently leading to delays in preparation for flight and fledging (Michaud et al, 2000). For a highly aerial species like purple martin, this could explain the large variation in fledge dates within nests but requires further investigation.

We found that the average date of nesting (first egg dates) of both ASY and SY parents were the same (ASY = 152 ± 1.44 , SY = 152.28 ± 1.04 days of the year). Previous studies showed later arriving SY female purple martins make their nest sooner after arrival than earlier birds, and do not have later first egg dates (Morton and Derrickson 1990). Therefore, while we did not measure spring arrival dates of adults, we did not expect that any influence of parental age on fledge date would be due to the later arrival date of SY parents at the breeding ground and later egg dates. Rather, due to the relative lack of experience and lower paternity assurance of first-time (SY) parents (Morton and Derrickson 1990, Tarof et al. 2011), we predicted fledge date of nestlings with SY parents to be later in comparison with nestlings of ASY parents which was supported by our results. Higher rates of extra-pair copulations between ASY males and SY females (Møller 1985), make it more probable that ASY males will be more successful than SY males in fertilizing eggs (Morton et al. 1990). Therefore, owing to reduced paternity assurance, SY males may decrease their paternal effort impacting the rate of nestling development (Morton and Derrickson 1990). Further, lower skill in foraging and feeding nestlings for younger females has been demonstrated in some studies, resulting in lower weight and slower growth of flight feathers of nestlings (e.g. Krist 2011, Bitton and Dawson 2017). Our results support these hypotheses, where SY males and females invest less in their offspring due to less certainty in paternity and skill in provisioning, respectively, which could be further explored to determine their relative influence on fledge dates.

In contrast with the impact of the number of nestlings on fledge date (Fig. 1A), juveniles from the same cavity (family groups) did not have similar colony departure timing (Supplemental Figure 2.4A). Instead, we observed that variation in fledge date for birds within the same cavity (0-4 days) (Supplemental Figure 2.2), became more variable by the time of colony departure (0-11 days). Previous studies reveal that timing can be highly heritable in songbirds, such as for migration timing in blackcaps (Sylvia atricapilla) (Pulido et al. 2001), spring arrival dates in barn swallows (Hirundo rustica) (Møller 2001), and moult timing in stonechats (Saxicola torquata) (Helm and Gwinner 1999) and garden warblers (Widmer 1999). The high within-nest variability in colony departure timing that we observed in this species could be related to high rates of extra-pair copulations resulting in mixed parentage within a nest (Morton et al. 1990), and consequently heritable differences in timing among nestmates (Pulido and Berthold 2003). Social impacts on timing may also have had an impact on withinnest variability. Separation of nestmates after fledging from their parents can result in 'adoption' by unrelated individuals in large colonial roosts that provide post-breeding provisioning (Brown 1978). In this case, joining different family groups could have a subsequent influence on departure timing, further increasing within-nest variability as birds depart the colony for the first time with new family groups.

Conclusion:

In a wild population of a migratory songbird, our results demonstrate an influence of nest timing (first egg date) on the timing of the first two stages of juvenile post-breeding movements (fledge date and colony departure date), where later nests resulted in later timing. However, the influence of nest timing weakened by colony departure date, likely through a combination of environmental and social factors. Family groups did not appear to depart colonies together, and adult movement timing was independent of the timing of nesting. Our results also revealed that both adults and juveniles departed their colonies within an average of two days of each other. Future experimental work would be best suited to further investigation of whether the timing of post-breeding movement is maintained across migration and in subsequent years.

Acknowledgment:

Funding was provided by the NSERC Discovery Grant Program, the University of Manitoba, Environment and Climate Change Canada (ECCC), and the Ontario Trillium Foundation. We thank Mike Cadman at ECCC and Stu Mackenzie and Tara Crewe of Birds Canada for fieldwork, as well as technical and other logistical support. We thank our field assistants Megan MacIntosh, Marine Morel, Brodie Badcock Parks, Alex Bencke, Aly Hyder Ali, and Vanessa Fiore. For other field assistance and onsite support, we thank Claude Radley and the Holiday Beach Bird Observatory crew, John Balga, Chris and Jennifer McKinnon, Ron Kingswood, Dave Smith, Kathryn Boothby, Nancy Furber, and Rick Ludkin.

References:

- Åkesson, S., and Hedenström, A. 2000. Wind selectivity of migratory flight departures in birds. BEHAV ECOL SOCIOBIOL. 47(3): 140-144.
- Allen, R. W. and Nice, M. M. 1952. A study of the breeding biology of the Purple Martin (Progne subis). AM MIDL NAT. 606-665.
- Baldwin, J. W., Leap, K., Finn, J. T., and Smetzer, J. R. 2018. Bayesian state-space models reveal unobserved offshore nocturnal migration from Motus data. ECOL MODEL. 386: 38-46.
- Bani Assadi, S., and Fraser, K. C. 2021. Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird. PROC. ROYAL. SOC. B, *288*(1957), 20211474.

Barton, K., and Barton, M. K. 2013. Package 'MuMIn'. Version, 1, 18.

- Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., and Dai, B. 2014. Linear mixedeffects models using Eigen and S4. R package version, 1(7).
- Berthold, P. 1993. Bird migration: a general survey. Oxford, Oxford University Press
- Berthold, P. 1996. Control of bird migration. Springer Science & Business Media.
- Berthold, P. 1988. The control of migration in European warblers. In XIX Congressus Internationalis Ornithologici (pp. 215-249). University of Ottawa Press.
- Bitton, P. P. and Dawson, R. D. 2017. Age-related prenatal maternal effects and postnatal breeding experience have different influences on nestling development in an altricial passerine. J AVIAN BIOL. 48(5): 660-668.
- Bojarinova, J., Ilves, A., Chernetsov, N. and Leivits, A. 2008. Body mass, moult and migration speed of the Goldcrest Regulus regulus in relation to the timing of migration at different sites of the migration route. Ornis Fennica. 85(2): 55-65.
- Both, C. 2010. Flexibility of timing of avian migration to climate change masked by environmental constraints en route. CURR BIOL. 20(3): 243-248.
- Brown, C. R. 1978. Post-fledging behavior of Purple Martins. Wilson Bull. 376-385.
- Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical informationtheoretic approach. New York Springer-Verlag.
- Coppack, T., Pulido, F. and Berthold, P. 2001. Photoperiodic response to early hatching in a migratory bird species. Oecologia. 128(2): 181-186.
- Coppack, T. and Pulido, F. 2004. Photoperiodic response and the adaptability of avian life cycles to environmental change. ADV ECOL RES. 35: 131-150.
- Cox, W. A., Thompson III, F. R., Cox, A. S. and Faaborg, J. 2014. Post-fledging survival in passerine birds and the value of post-fledging studies to conservation. J WILDLIFE MANAGE. 78(2): 183-193.
- Crewe, T. L., Crysler, Z. and Taylor, P. 2018. Motus, R. A walk through the use of R for Motus automated radiotelemetry data; https:// motus.org/MotusRBook/index.html (accessed Feb 26, 2018).
- Deakin, J. E., Guglielmo, C. G. and Morbey, Y. E. 2019. Sex differences in migratory restlessness behavior in a Nearctic–Neotropical songbird. Auk, 136(3), ukz017.
- De Frutos, A. and Olea, P. P. 2008. Importance of the premigratory areas for the conservation of lesser kestrel: space use and habitat selection during the post-fledging period. ANIM CONSERV. 11(3): 224-233.
- Dellinger, T. B. and Rogillio, C. 1991. The graphic development of baby Purple Martins. *Purple Martin Update*. 3(1):6-11.
- De Sante, D. F., Saracco, J. F., de Vivar Alvarez, C. R. and Morales, S. 2009. Instructions for establishing and operating bird-banding stations as part of the MoSI program. *Institute for Bird Populations, Pt. Reyes Station*.
- Ellegren, H. 1990. Timing of autumn migration in Bluethroats Luscinia s. svecica depends on timing of breeding. Ornis Fenn. 67: 13-17.
- Fraser, K. C., Shave, A., Savage, A., Ritchie, A., Bell, K., Siegrist, J., Ray, J.D., Applegate, K. and Pearman, M. 2017. Determining fine-scale migratory connectivity and habitat selection for a migratory songbird by using new GPS technology. J. Avian Biol, 48(3), 339-345.
- Freed, L. A. 1988. Forced fledging-and investigation of the lengthy nestling period of tropical house wrens. Natl Geogr Res. 4(3): 395-407.
- Gagné, M. 2019. The effect of artificial light at night (ALAN) on the growth and migration timing of purple martins (Progne subis) (Unpublished honours Thesis). University of Manitoba, Winnipeg, Manitoba, Canada.
- Gómez, C., Bayly, N. J., Norris, D. R., Mackenzie, S. A., Rosenberg, K. V., Taylor, P. D., Hobson, K.A. and Cadena,C. D. 2017. Fuel loads acquired at a stopover site influence the pace of intercontinental migration in a boreal songbird. SCI REP. 7(1): 1-11.
- Grüebler, M. U. and Naef-Daenzer, B. 2010. Survival benefits of post-fledging care: experimental approach to a critical part of avian reproductive strategies. J ANIM ECOL. 79(2): 334-341.
- Gwinner, E. 1986. Circannual rhythms in the control of avian migrations. Adv Study Behav. Vol (16): 191-228.
- Gwinner, E. 1996a. Circadian and circannual programmes in avian migration. J EXP BIOL. 199(1): 39-48.
- Gwinner, E. 1996b. Circannual clocks in avian reproduction and migration. Ibis. 138(1): 47-63.
- Gwinner, E. 2003. Circannual rhythms in birds. CURR OPIN NEUROBIOL. 13(6): 770-778.

- Gwinner, E. 1989. Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. J BIOL RHYTHM. 4:237–250
- Hall, K. S. S. and Fransson, T. 2000. Lesser Whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. J AVIAN BIOL. 31(4): 583-587.
- Hazra, P., Sinha, A., Mondal, P. and Khan, T. N. 2012. Calendar-effects and temperature-impacts in migratory waterbirds at three tropical Indian wetlands. ACTA OECOL, 43, 60-71.
- Helm, B., and Gwinner, E. 2006. Migratory restlessness in an equatorial nonmigratory bird. PLoS Biol, 4(4), e110.
- Helm, B., and Gwinner, E. 1999. Timing of postjuvenal molt in African (Saxicola torquata axillaris) and European (Saxicola torquata rubicola) stonechats: effects of genetic and environmental factors. Auk. 116(3): 589-603.
- Helm, B., Van Doren, B. M., Hoffmann, D. and Hoffmann, U. 2019. Evolutionary response to climate change in migratory pied flycatchers. CURR BIOL. 29(21): 3714-3719.
- Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T. and Bloch, G. 2017. Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. PHILOS T ROY SOC B. 372(1734): 20160246.
- Jenni, L. and Winkler, R. 1994. Moult and ageing of European Passerines. London: Academic Press. 225 p.
- Kershner, E. L., Walk, J. W. and Warner, R. E. 2004. Post-fledging movements and survival of juvenile Eastern Meadowlarks (Sturnella magna) in Illinois. Auk. 121(4): 1146-1154.
- Knudsen, E., Lindén, A., Both, C., Jonzén, N., Pulido, F., Saino, N., Sutherland, W.J., Bach, L.A., Coppack, T., Ergon, T. and Gienapp, P. 2011. Challenging claims in the study of migratory birds and climate change. BIOL REV. 86(4): 928-946.
- Krist, M. 2011. Egg size and offspring quality: a meta-analysis in birds. BIOL REV. 86(3): 692-716.
- Michaud, T., and Leonard, M. 2000. The role of development, parental behavior, and nestmate competition in fledging of nestling tree swallows. Auk, 117(4): 996-1002.
- Mitchell, G. W., Newman, A. E., Wikelski, M. and Ryan Norris, D. 2012. Timing of breeding carries over to influence migratory departure in a songbird: an automated radiotracking study. J ANIM ECOL. 81(5): 1024-1033.
- Møller, A. P. 2001. Heritability of arrival date in a migratory bird. Proc. Royal Soc. B. 268(1463): 203-206.
- Møller, A. P. 1985. Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow Hirundo rustica. BEHAV ECOL SOCIOBIOL. 17(4): 401-408.
- Morton, E. S., and Derrickson, K. C. 1990. The biological significance of age-specific return schedules in breeding Purple Martins. Condor. 92(4): 1040-1050.

- Morton, E. S., Forman, L. and Braun, M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. Auk. 107(2): 275-283.
- Morton, M.L. and Pereyra, M.E. 1994 Autumnal migration departure sched- ules in mountain white-crowned sparrows. Condor. 96: 1020–1029.
- Naef-Daenzer, B. and Grüebler, M. U. 2016. Post-fledging survival of altricial birds: Ecological determinants and adaptation. J FIELD ORNITHO. 87(3): 227-250.
- Newton, I. 2010. The migration ecology of birds. Elsevier.
- Newton, I. 2011. Migration within the annual cycle: species, sex and age differences. J. Ornithol. 152(1): 169-185.
- Noskov, G. A., Rymkevich, T. A. and Iovchenko, N. P. 1999. Intraspecific variation of moult: adaptive significance and ways of realisation. InProc. Int. Ornithol. Congr. Vol (22): 544-563.
- Ouwehand, J., Burger, C., and Both, C. 2017. Shifts in hatch dates do not provide pied flycatchers with a rapid ontogenetic route to adjust offspring time schedules to climate change. Funct Ecol. 31(11), 2087-2097.
- Pulido,F. and Berthold, P. 2003. Quantitative genetic analysis of migratory behavior. In Avian migration (eds. P. Berthold,E. Gwinner&E. Sonnenschein), pp. 53–77. Springer, Berlin, Germany.
- Pulido, F., Berthold, P., Mohr, G. and Querner, U. 2001. Heritability of the timing of autumn migration in a natural bird population. Proc. Royal Soc. B. Series B. 268(1470): 953-959.
- Pulido, F. and Widmer, M. 2005. Are Long-Distance Migrants Constrained in Their Evolutionary Response to Environmental Change? Causes of Variation in the Timing of Autumn Migration in a Blackcap (S. atricapilla) and Two Garden Warbler (Sylvia borin) Populations. ANN NY ACAD SCI. 1046(1): 228-241.
- Pyle, P. 1997. Identification guide to North American birds: a compendium of information on identifying, ageing, and sexing" near-passerines" and passerines in the hand. Slate Creek Press.
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <u>http://www.R-project.org/.</u>
- Rappole, J. H. and Tipton, A. R. 1991. New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). J FIELD ORNITHOL. 335-337.
- Remeš, V. 2007. Avian growth and development rates and age-specific mortality: the roles of nest predation and adult mortality. J. Evol. Biol. 20(1): 320-325.
- Sick, H. 1993. Birds in Brazil. Princeton University Press, Princeton, NJ.
- Sokolov, L. V. 2000. Spring ambient temperature as an important factor controlling timing of arrival, breeding, postfledging dispersal and breeding success of Pied Flycatchers Ficedula hypoleuca in Eastern Baltic.

- Tarof, S. A., Kramer, P. M., Hill III, J. R., Tautin, J. and Stutchbury, B. J. 2011. Brood size and late breeding are negatively related to juvenile survival in a Neotropical migratory songbird. Auk. 128(4): 716-725.
- Taylor, P.D., Crewe, T.L., Mackenzie, S.A., Lepage, D., Aubry, Y., Crysler, Z., Finney, G., Francis, C., Guglielmo,C., Hamilton, D. and Holberton, R.L. 2017. The Motus Wildlife Tracking System: a collaborative researchnetwork to enhance the understanding of wildlife movement. AVIAN CONSERV ECOL. 12(1).
- Vitz, A. C. and Rodewald, A. D. 2011. Influence of condition and habitat use on survival of post-fledging songbirds. Condor. 113(2): 400-411.
- Wagner, R. H., Schug, M. D. and Morton, E. S. 1996. Confidence of paternity, actual paternity and parental effort by purple martins. Anim Behav: 52(1): 123-132.
- Widmer, M. 1999. Altitudinal variation of migratory traits in the Garden Warbler Sylvia borin. PhD thesis, University of Zurich, Switzerland

Table 2.1: LMM analysis to explain variation in timing of fledge date and colony departure date of juveniles and adult purple martins. The fixed effects were first egg date, number of nestmates, age of parents, weight of nestlings and fat score. The cavity ID was the random effect. This table shows the best supported candidate models for each. The full set of candidate models are presented in supplementary tables 2.1-2.2.

Model	Parameter	Estimat e	Std. Error	95% CI (lower)	95% CI (upper)	AICc	W
Fledge date	First egg date	1.03	0.08	0.87	1.20		
(Juveniles)	Parent age (SY)	1.79	0.81	0.19	3.38	244	0.341
	Number of nestmates	1.13	0.36	0.42	1.83		
		Variance	SD	%Variance			
	Cavity (random factor)	1.37	1.17	44.95			
	Residual	1.687	1.29				
Colony	First egg date	0.52	0.26	0.00	1.04	2 55.1	0.339
departure date							
(Juveniles)		Variance	SD	%Variance			
	Cavity (random factor)	15.05	3.88	59.04			
	Residual	10.44		3.23			
Colony departure date (Adults)	Null					129.2	0.544
		Variance	SD	%Variance			
	Cavity (random factor)	12.18	3.49	55.95			
	Residual	9.58	3.09				



Figure 2.1 A&B: 95% confidence interval from the model (LMM) estimate of the variables for fledge date for juvenile purple martins. The red bar shows estimate ± 1.96 *se, and the black bar color shows estimate \pm se (A). The correlation between first egg date and fledge date, gray shading shows the confidence interval (B).



Figure 2.2 A-D: 95% confidence interval from the model (LMM) estimate of the variables for colony departure date of juvenile (A) and adult purple martins (B). The red bar color shows an estimate ± 1.96 *se, and the black bar color shows an estimate \pm se (A & B). The correlation between first egg date and colony departure date of juveniles (C) and adults (D), gray shading shows the confidence interval (C).



Figure 2.3: The correlation between first egg date and age at colony departure date, grey shading shows 95% confidence interval



Figure 2.4: Comparison of variation of fledge date and colony departure date among nestmates

Chapter 3: Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird

Saeedeh Bani Assadi1 and Kevin Charles Fraser2

1University of Manitoba, Winnipeg, Canada R3T 2N2

2University of Manitoba Ringgold standard institution, Winnipeg, Canada

Status: published in Proceedings of the Royal Society B (https://doi.org/10.1098/rspb.2021.1474)

Abstract:

Previous lab studies have demonstrated the role of photoperiod in cueing the migration timing of small land birds, however, how migration timing of young birds in wild environments develops in relation to these cues has rarely been investigated. Such investigations can make important contributions to our developing understanding of the phenotypic plasticity of migration timing to new conditions with climate change, where changes in the timing of nesting may expose juvenile birds to different photoperiods. We investigated the impact of manipulating photoperiod during nestling development in a long-distance migratory songbird on the timing of post-breeding movements in the wild. Using programmable lighting installed in the nest boxes of purple martin (Progne subis), we exposed developing nestlings, from hatch to fledge date, to an extended photoperiod that matched the daylength of the summer solstice in Manitoba, Canada. We found that birds with a simulated, earlier photoperiod had a longer nesting period, and later fledge and fall departure dates than control group birds. This study demonstrates the phenotypic plasticity of first-year birds to the ontogenetic effect of their hatch date in the formation of the timing of their first post-breeding movements. Further, we discuss how these results have implications for the potential use of assisted evolution approaches to alter migration timing to match new conditions with climate change.

Keywords: photoperiod, phenotypic plasticity, post-breeding movements, ontogenetic effect, assisted evolution

Introduction:

Spring advancement due to climate change has resulted in different phenological responses by migratory birds, such as shifts in their migration phenology, or the timing of other seasonal events such as breeding [1]. Among migratory birds, long-distance migrants may be more dependent on their endogenous, circannual schedules to cue their migration departure timing [2,3] as they cannot predict phenological advancement at their breeding ground from overwintering areas that may be thousands of kilometres away [4]. Yet, breeding arrival timing has been observed in many long-distance migratory species to have advanced over years or decades. However, the mechanisms for these changes have been much debated and require further exploration (e.g. [5, 6])

Migration timing of long-distance migratory birds is mainly controlled by endogenous circannual rhythms synchronized to the external cue of photoperiod [2,3,7]. With climate change and corresponding advancing springs, birds that breed earlier can be exposed during nesting to different day lengths (photoperiod), which may further influence timing. Phenotypic plasticity may be a mechanism by which individuals adaptively respond to new environmental conditions [8, 9], and that could provide long-distance migrants with a shorter-term mechanism for adapting to climate change. A study by Both [10] using band recapture data for European pied flycatchers (*Ficedula hypoleuca*) found that breeding latitude predicted timing of median recovery date during spring migration at stopover sites. It was proposed that these patterns were driven by the different day lengths experienced by nestlings at more northern versus more southern breeding latitudes. This led to the inference that migration timing may be flexible to past experiences at the breeding grounds, based upon an ontogenetic effect of photoperiod during nestling development [10]. This is supported by a laboratory study by Coppack *et al.* [11] on European blackcaps (*Sylvia atricapilla*) showing that simulating an earlier photoperiod during the nestling phase, resulted in a longer moulting

period and later onset of fall migration. In a rare, experimental field study, delaying the hatch date of pied flycatchers through manipulation of incubation timing resulted in a later spring arrival date in the following spring [12]. However, further study is required in other systems, particularly where photoperiod is manipulated, and the actual movements of individuals are directly tracked in the wild. Indeed, new syntheses of chronobiology and ecology through a 'wild clock' approach in a greater variety of study systems are predicted to yield invaluable new insights into how animal timing responds to environmental change [1, 13]. Such research would complement previous laboratory and field research to address the current knowledge gap as to whether birds experiencing different photoperiods with earlier nests in advanced springs provide new experiences of zeitgebers that entrain an adaptive response to climate change (ontogenetic effect) in migratory songbirds.

In this study, we build upon laboratory studies by using a wild system to investigate the direct impacts of experimentally manipulated photoperiod on the development of timing in a long-distance migratory songbird. We aimed to experimentally test the hypothesis that the photoperiod experienced by juveniles in the nest during development impacts the timing of their subsequent lifecycle events. Using programmable lights installed in the nest boxes of purple martins (*Progne subis*) during the nestling phase, we manipulated photoperiod to summer solstice day length to simulate an earlier calendar date, as the hatch date of many birds occurs naturally after this date and birds experience shorter day length. The other environmental factors were the same for all nestlings.

We tagged nestlings and used an automated telemetry system [14] to determine subsequent migration departure dates for experimentally manipulated nestlings and controls. We predicted that nestlings exposed to extended day lengths (photoperiod) simulating earlier calendar dates would have a longer nesting period (pre-fledge) and would spend more time at the colony post-fledge resulting in later fall departure dates.

Methods:

We conducted the field component of this study at three purple martin breeding colonies in southern Manitoba, Canada (site one: 49.734° N, 97.1317° W, site two: 49.127° N, 97.5703 W and site three: 50.173442° N, 97.133442° W) which are located at open habitats close to water bodies. This colonial bird is dependent on human-made houses for breeding in this part of its range. The number of purple martin houses at sites one, two and three were three units (with 14 nest cavities each), 4 units (with 32, 12, 14 and 8 cavities each) and two units (with 14 nest cavities each), respectively. Purple martins are aerial insectivores that breed in eastern North America and journey 10-20,000 km annually to overwintering locations mostly in the Amazon Basin in Brazil [15]. Spring migration starts in mid-April for our study population and arrival and departure date at these latitudes is early May and mid-August, respectively [16, 17]. To manipulate photoperiod to test for its impact on the timing of fledge and post-breeding movements, we mounted programmable light units inside treatment nest boxes. We installed small, light-emitting diodes (LED) (5mm length) in the roof of each nest cavity so that they pointed downward toward the nest [18,19]. We selected LEDs because they incorporate the spectrum of natural light, do not produce heat [19, 20], and can be programmed to emit selected amounts (lux) of light. Each light was connected by a thin wire to an external unit composed of an Arduino and a real-time clock (RTC), mounted on a circuit board. Each of the 4 units was connected to lights in 5 cavities and was mounted underneath the housing units along with a rechargeable battery. In total, we used 20 nest cavities for this manipulation. The same number of nest cavities were used as the control group at two breeding sites.

We programmed the LEDs to emit 1.5 lux, which was the average (n=4) measured at the start of civil twilight before sunrise at our study areas (Digital illuminance meter, LX 1330B). To experimentally extend day length, lights were programmed to match the day length of the

summer solstice (21 Jun 2019, 16:21:06) at our study sites. We programmed Arduino units to turn the light on one hour before sunset and off at the end of the summer solstice civil twilight. In the morning, lights turned on again according to the summer solstice civil twilight and off one hour after sunrise. Most hatch dates at our study sites were just after the summer solstice [12] when days were getting shorter, so we opted to 'hold' photoperiod at the longest day of the year (the solstice) for experimental cavities to simulate an earlier photoperiod for all nests (Supplemental Fig. 3.1). We simulated the photoperiod of the summer solstice (21 June), which is the longest day of the year because 1) at more northern latitudes most individuals hatched after the summer solstice, 2) it is hypothesized that this time of the year could be a baseline to re-set internal clock time [21], and 3) the timing of the summer solstice in relation to nesting provides the best opportunity to manipulate the photoperiod in purple martin cavities during nesting in a wild environment (i.e. extending day length for nestlings once day lengths are getting shorter).

To track fledge dates and post-breeding movements, we used the Motus Wildlife Tracking System (www.motus.org) which is an international, automated radio-telemetry array of receiving stations [14]. We randomly selected four nestlings from each of the extended day length and control nest cavities when they were near fledging (age 20-22 days). We outfitted each with individually-coded, radio nanotags (NTQB-3-2, Lotek Inc.) (0.67 g, 12 x 6 x 5 mm in length, width and height, respectively) using a leg-loop, backpack style harness [22] made of polypropylene thread. The average weight of nestlings near their fledge date is 50 grams [23, 24] and the weight of the tags we used is, therefore, less than 3% of their total body weight. Each tag emitted a signal every 10 seconds and had a battery life of approximately 124 days. A total of 80 nestlings from each of the extended day length and control groups were equipped with tags. We weighed each nestling at the time of tagging and their fat content (scored from 0-5, following MoSI protocol [25]) was also determined. At

each site, we set up a Motus receiver within ~ 8-70 meters to the purple martin houses. Each receiver tower included one omnidirectional antenna with an approximate detection range of 500 m and two 9-element Yagi antennas with a range of approximately 15 km [14].

We determined fledge date for each nestling based on variation in the signal strength of the nanotags and observations from frequent nest checks. Signal strength varied little while nestlings were within their cavities at a constant distance from the receiver but began to fluctuate widely at the time of fledging when the distance between tag and receiver shifted rapidly with first flights from the nest and continued to vary more widely thereafter (Supplementary Fig. 3.2). We conducted nest checks every other day at each colony and could confirm from visual observations of the nest that young in a nest had fledged. To determine the timing of colony departure, we used the presence of a fading signal and subsequent end of detection of individual tag signals to indicate the day that a bird had departed (Supplementary Fig. 3.2).

All data collection procedures and experiments were conducted in accordance with the guidelines of the University of Manitoba's Animal Care Committee who have approved this project (Animal Care Protocol Number F18-031/1(AC11388)).

Data analysis:

We used linear mixed-effects models (LMM) fit by REML from the package *lme4* [26] to assess the influence of experimentally extended day length on the timing of fledge date, nesting period, duration at the colony post-fledge, and colony departure date. The fixed variables were first egg date, number of nestmates in each cavity, and treatment (experimentally extended day length). The first egg date factor could account for the photoperiod that nestlings experienced as well as other within-season variations, such as the amount of available food. In addition to these fixed variables, weight and fat score at the date of tagging the birds were also considered in the models of nesting period and fledge date.

Nested random effects of cavity ID and colony site were set to control for their variations such as potential variability associated with the level of cavities and sites through the season. However, likelihood ratio tests showed the random effect of site was not significant for our models (χ^2 =0, *P*=1), therefore for parsimony the random effect of site was removed from the analysis. The interactions between treatment and first egg date and treatment and weight were examined in the models, but preliminary analysis showed these did not have significant impacts and therefore they were removed from the global model. The normality of residual distribution of the models was tested and any statistical outliers on the basis of Cook's D were removed from the dataset. Akaike Information Criteria corrected for small sample size (AICc) [27] using the package "MuMIn" [28] was used to run all possible candidate models that could be built from the full model. Among all competitive models with Δ AICc<2, we used the Akaike weight (*w*) to select the most parsimonious model [27]. All analyses were conducted in R version 3.6.1. [29].

Results:

Among the tagged nestlings, we could track the fledge date of 51 individuals from the control group and 68 from the treatment (extended day length) group. Of these, we determined 30 colony departure dates for control birds and 49 departure dates for extended day length birds.

Nesting period and fledge date:

The average duration of the nesting period for control and extended day length groups was 28 ± 0.00 and 29.69 ± 0.22 , respectively (Fig. 1). Nestlings in the extended day length group had a nesting period that was 1.05 ± 0.36 (95% CI -1.76 to -0.33) days longer than birds in the control group. The number of nestlings also impacted duration in the nest, where one more nestmate resulted in 0.37 ± 0.15 days (95% CI 0.06 to 0.68) longer in the nest (Table 1).

The fledge date of all juveniles was between July 12^{th} and August 1^{st} . The mean date of fledging for juveniles in the extended day length group was July 23^{rd} (204 ± 0.53), while birds in the control group fledged on average on July 21^{st} (202.96 ± 0.64). The experimental, extended day length treatment had an impact on fledge date with a 1.37 ± 0.66 day (95% CI - 2.66 to -0.07) delay as compared to birds in the control group (Table 1). First egg date had the largest influence on fledge date as expected, where for every one-day delay in first egg date, fledge date was 0.97 ± 0.07 days (95% CI 0.82 to 1.12) later (Fig. 3a; Table 1).

Duration at colony post-fledge and departure date:

The juveniles in the experimental, extended day length group spent more time at the colony post-fledge than those that experienced natural photoperiod only (average of 4 ± 0.00 versus 5 ± 0.33). However, the best model only included first egg date as a predictor variable of colony duration (Table 1, Fig. 2). All tagged birds departed the colony between July 16^{th} and August 9^{th} . The mean departure date for juveniles in the control group was July 28^{th} (208.8±0.92) and July 29^{th} (209.95±0.51) for birds in the extended day length group. As expected, first egg date had an important and positive relationship with colony departure date (0.90±0.09, 95% CI 0.70 to 1.09) (Table 1, Fig. 3b). However, juveniles exposed to an experimentally extended day length in the nest box during development had colony departure dates that were 2.38 ± 0.88 days later (95% CI -4.10 to -0.65) than juveniles that were exposed to natural day lengths only (Table 1, Fig 3b). This relationship changed over the colony departure period, where birds with a later departure date were more similar to controls (Fig 3b).

Discussion:

Our study experimentally investigated the potential ontogenetic role of photoperiod in the development of post-breeding movement timing of free-living, long-distance migratory songbirds (Supplemental Fig. 3.3). We show that exposing nestlings to an extended (earlier)

photoperiod in a wild environment resulted in a delay in fledge date and colony departure date as compared to birds that experienced natural photoperiod (Table 1). These results demonstrate phenotypic plasticity in the timing of the post-fledge movements of nestling songbirds in response to day length experienced in the nest.

Our results are consistent with, and complement, some prior lab-based experiments investigating the role of photoperiod in seasonal timing [30, 31, 32]. For example, Coppack *et al.* [11] showed prolongation of moulting and a delay in migration timing (measured through the proxy of *Zugunruhe*) of European blackcap juveniles in response to a lab-based simulation of an earlier photoperiod during nestling development. Our field-based experiment demonstrates that the extension of photoperiod during nestling development carries over to have an impact on colony-departure decisions in free-living birds, directly tracked during actual movements. Even a small shift in egg-laying date and consequently fledge date, particularly at more northern latitudes [33] can potentially have carryover effects on the survival of young of aerial insectivores [34] and their subsequent life cycle events [10].

Our results are also consistent with, and help to explain, inferences from field-based, correlational studies of Arctic terns (*Sterna paradisea*) and pied flycatchers, where breeding date influenced the timing of subsequent migration [10, 35]. For pied flycatchers, advances in the recovery dates of banded birds during spring migration were attributed to an ontogenetic effect of advancing breeding dates at more northern breeding latitudes in the previous season [10]. In another study of pied flycatchers, natal nest timing carried over to influence arrival date and breeding in the subsequent year, for 3 of 5 years examined [12]. Similarly, advancing breeding dates in one season correlated with advanced arrival and breeding dates in the subsequent season in Arctic terns. These were attributed to a plastic ("learning") effect of timing that carried over from the previous year [35]. Our experimental results align with these observations and illustrate that photoperiod during development may be an important

mechanism underlying these advances in timing. Taken together, these results suggest that the timing of seasonal behaviours is generally sensitive to the day length experienced while birds are developing within the nest, but that variation may be introduced or timing can be constrained by other endogenous or exogenous factors [10, 12, 36]. Moreover, the manipulation of light in our experiment could be considered to mimic the day length (summer solstice) of a more northern latitude for the period of the experimental treatment, where the later timing of birds in our experiment may reflect a natural adjustment to breeding at different latitudes. According to a study by Both (2010), variation in the timing of pied flycatchers originating from more northern in comparison with more southern nests was attributed to the experience of different photoperiod regimes at different latitudes [10].

Our results also indicate further flexibility in timing post-fledge, in that the experimental treatment had a differential impact on early versus later hatching birds. The steep slope of the correlation between colony departure date and first egg date of both groups showed that young of the treatment group from later nests had earlier departure dates relative to their hatching dates (Fig 3b). Seasonally decreasing day lengths post-fledge can have the influence of speeding up the development of later-hatched young; a so-called 'calendar effect' [37], which may be an adaptation so that later hatching birds can prepare for autumn migration (e.g. [38, 39, 40, 41]). The later-hatching birds in our experimental group would have experienced the largest shift in day length between the experimentally extended day length within the nest box (held to summer solstice) and the shorter, more rapidly decreasing natural day length they experienced once they fledged from their nest boxes. We infer that the earlier departure of these later hatching birds relative to their hatch date may correspond to a stronger 'calendar' effect induced by this larger shift in photoperiod experienced by these individuals, as compared to birds in the control group. Photoperiodic cues indicating that birds may be 'late' may induce a stronger migratory response. Long-tailed tits (*Aegithalos c*.

caudatus) intercepted during migration and exposed in the lab to a photoperiod simulating one month later had a stronger migratory response (measured via Zugunruhe) than birds exposed to natural day lengths [42].

It is not known whether the impacts of photoperiod on timing that we measured would impact timing over the rest of the calendar year, or further into adulthood. Or, if the ontogenetic effect of photoperiod experienced during nesting is swamped by other intervening influences. This is important, as a phenotypic advancement in the timing of nesting in response to climate change could translate to rapid adjustment to climate change effects if the timing is carried into later life stages [10]. In pied flycatchers, a one-week experimental delay in hatch date led to the ontogenetic effect of later spring arrival and egglaying dates only for the first subsequent year but did not continue to influence timing in the subsequent one to two years [12]. Similarly, in a different study of pied flycatchers, there was no influence of hatch date on the timing of birds after the juvenile stage [43]. The fact that our experimental birds that fledged later tended to be closer in colony departure timing to controls, suggests further plasticity after the experimental treatment to ambient light conditions. However, future investigation of how long the influence of photoperiod in the nest predicts the timing of adult birds is required.

Further, we found that brood size impacted the duration of the nesting period, where larger broods resulted in longer nesting time. It has been demonstrated that greater brood size results in greater competition for food in purple martins, therefore prolonging the time required to reach the appropriate condition for fledging [44].

Assisted evolution approaches are being applied in coral reef systems, where corals are pre-adapted to current and predicted increases in ocean temperature [45]. Assisted evolution approaches that address mismatches between environmental phenology and migratory bird timing may be desirable [46], in response to the precipitous declines in the North American

avifauna in recent decades [47]. Our results suggest that a manipulation of day length (simulating an earlier calendar date) from hatch to fledge can delay departure date of nestlings and sheds some light on how an assisted evolution approach could potentially be used to shift the timing of migration based on manipulations in the nest. However, once exposed to natural photoperiod after fledging, it appeared that birds in the experimental group continued to shift, suggesting that timing may not be fixed and will continue to change post-fledge. Future studies could focus on how long timing shaped in the nest may carry post-fall migration and/or whether there is a period after which the timing routines of young birds become more 'fixed'. Such studies could help to further reveal whether an assisted evolution approach to phenological mismatch, where birds raised in captivity and released in the wild are 'instilled' with more adaptive timing, may be a viable method.

Conclusion:

Our study demonstrates the ontogenetic effect of day length experienced in the nest on the subsequent post-breeding movement timing of juvenile, migratory songbirds in the wild. Climate change is rapidly altering environmental phenology resulting in earlier springs and correspondingly earlier nest dates [10] which can expose nestling migratory birds to different photoperiods. Our results demonstrate that the manipulation of photoperiod experienced by nestlings in the wild influences the subsequent timing of their movements. Future research could further investigate the efficacy of similar manipulations as part of an assisted evolution approach to timing mismatches in wild songbirds.

Acknowledgements

Funding was provided by a John R. Evans Leaders Fund from the Canadian Foundation for Innovation, Research Manitoba, the Natural Sciences and Engineering Research Council's Discovery Grant Program, and the University of Manitoba. We thank Christie Lavallée, Evelien de Greef, and Leanne Neufeld for their assistance with fieldwork. We thank Gail

Davoren, Colin Garroway, and Saman Muthukumarana for helpful comments on earlier drafts of this manuscript. We thank Kristian Melo for designing, building and programming the LED light apparatuses. We especially thank purple martin colony managers Alan Enns and Paul and Maxine Clifton for their patience and assistance with this research.

References:

 Åkesson S, Ilieva M, Karagicheva J, Rakhimberdiev E, Tomotani B, Helm B. 2017 Timing avian long-distance migration: from internal clock mechanisms to global flights. *Phil. Trans. R. Soc. B* 372 (1734):20160252. (http://dx.doi.org/10.1098/rstb.2016.0252)

2. Berthold P. 1996 Control of bird migration. Springer Science & Business Media.

Gwinner E. Circadian and circannual programmes in avian migration. 1996 J Exp Biol. 199(1):39 48. (https://doi.org/10.1242/jeb.199.1.39)

 Both C, Visser ME. 2001 Adjustment to climate change is constrained by arrival date in a longdistance migrant bird. *Nature*.411(6835):296-8. (<u>https://doi.org/10.1038/35077063</u>)

Knudsen E, Lindén A, Both C, Jonzén N, Pulido F, Saino N, Sutherland WJ, Bach LA, Coppack
 T, Ergon T, Gienapp P. 2011 Challenging claims in the study of migratory birds and climate change. *Biol Rev.* 86(4):928-46. (https://doi.org/10.1111/j.1469-185X.2011.00179.x)

Gill JA, Alves JA, Sutherland WJ, Appleton GF, Potts PM, Gunnarsson TG. 2014 Why is timing of bird migration advancing when individuals are not?. *Proc. R. Soc. B* 281(1774):20132161.
 (http://dx.doi.org/10.1098/rspb.2013.2161)

 Gwinner E. 1972 Adaptive functions of circannual rhythms in warblers. InXVth Congressus Internationalis Ornithologici (pp. 218-236). EJ Brill.

8. Price TD, Qvarnström A, Irwin DE. 2003 The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. B.* 270(1523):1433-40. (https://doi.org/10.1098/rspb.2003.2372)

9. Yeh PJ, Price TD. 2004 Adaptive phenotypic plasticity and the successful colonization of a novel environment. *The Am Nat*. 164(4):531-42. (https://doi.org/10.1086/423825)

10. Both C. 2010 Flexibility of timing of avian migration to climate change masked by environmental constraints en route. *Curr Biol.* 20(3):243-8. (https://doi.org/10.1016/j.cub.2009.11.074)

 Coppack T, Pulido F, Berthold P. 2001 Photoperiodic response to early hatching in a migratory bird species. *Oecologia*. 128(2):181-6. (https://doi.org/10.1007/s004420100652)

Ouwehand J, Burger C, Both C. 2017 Shifts in hatch dates do not provide pied flycatchers with a rapid ontogenetic route to adjust offspring time schedules to climate change. Funct Ecol.(11):2087-97. (https://doi.org/10.1111/1365-2435.12940)

Helm B, Visser ME, Schwartz W, Kronfeld-Schor N, Gerkema M, Piersma T, Bloch G. 2017 Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. *Phil. Trans. R. Soc. B*. 372(1734):20160246. (http://dx.doi.org/10.1098/rstb.2016.0246)

14. Taylor P, Crewe T, Mackenzie S, Lepage D, Aubry Y, Crysler Z, Finney G, Francis C, Guglielmo C, Hamilton D, Holberton R. 2017 The Motus Wildlife Tracking System: a collaborative research network to enhance the understanding of wildlife movement. *Avian Conserv Ecol.* 12(1).

(https://doi.org/10.5751/ACE-00953-120108)

15. Fraser KC, Stutchbury BJ, Silverio C, Kramer PM, Barrow J, Newstead D, Mickle N, Cousens BF, Lee JC, Morrison DM, Shaheen T. 2012 Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. *Proc. R. Soc. B* 279(1749):4901-6. (https://doi.org/10.1098/rspb.2012.2207)

 Lavallée CD, Assadi SB, Korpach AM, Ray JD, Fischer JD, Siegrist J, Fraser KC. 2021 The use of nocturnal flights for barrier crossing in a diurnally migrating songbird. *Mov. Ecol.* 9(1):1-1.
 (https://doi.org/10.1186/s40462-021-00257-7)

17. Neufeld LR, Muthukumarana S, Fischer JD, Ray JD, Siegrist J, Fraser KC. 2021 Breeding latitude is associated with the timing of nesting and migration around the annual calendar among Purple Martin (Progne subis) populations. *J Ornithol.* 18:1-6. (https://doi.org/10.1007/s10336-021-01894-w)

18. Raap T, Casasole G, Pinxten R, Eens M. 2016a Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds. *Environ Pollut*. 218:909-14. (https://doi.org/10.1016/j.envpol.2016.08.024)

19. Raap T, Casasole G, Costantini D, AbdElgawad H, Asard H, Pinxten R, Eens M. 2016b Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study. *Sci Rep.* 6:35626. (https://doi.org/10.1038/srep35626)

Schubert EF, Kim JK. 2005 Solid-state light sources getting smart. *Science*. 308(5726):1274-8.
 (10.1126/science.1108712)

21. Newton I. 2010 The migration ecology of birds. Elsevier

Rappole JH, Tipton AR. 1991 New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). *J Field Ornithol*.
 335-7. (<u>https://www.jstor.org/stable/20065798</u>)

Allen RW, Nice MM. 1952 A study of the breeding biology of the Purple Martin (Progne subis).
 Am. Midl. Nat. 606-65. (https://doi.org/10.2307/2422034)

24. Dellinger TB, Rogillio C. 1991 The graphic development of baby Purple Martins. *Purple Martin Update*. 3(1):6-11.

25. De Sante DF, Saracco JF, de Vivar Alvarez CR, Morales S. 2009 Instructions for establishing and operating bird-banding stations as part of the MoSI program. *Institute for Bird Populations, Pt. Reyes Station.*

Bates D, Maechler M, Bolker B, Walker S. lme4: Linear mixed-effects models using Eigen and
 S4. R package version 1.1-7

27. Burnham KP, Anderson DR. 2002 A practical information-theoretic approach. Model selection and multimodel inference, 2nd ed. Springer, New York.

Barton K. MuMIn: Multi-Model Inference. 2019 R package version 1.43.15. <u>https://CRAN.R-project.org/package=MuMIn</u>.

Team RC. 2014 R: A language and environment for statistical computing (version 3.1. 2). Vienna,
 Austria. R Foundation for Statistical Computing.

30. Gwinner E. 1989 Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. *J Biol Rhythm*.4(2):125-38. (https://doi.org/10.1177/074873048900400210)

31. Coppack T, Pulido F. 2004 Photoperiodic response and the adaptability of avian life cycles to environmental change. *Adv Ecol Res.* 35:131-50. (https://doi.org/10.1016/S0065-504(04)35007-5)

32. Coppack T, Tindemans I, Czisch M, Van der Linden A, Berthold P, Pulido F. 2008 Can longdistance migratory birds adjust to the advancement of spring by shortening migration distance? The response of the pied flycatcher to latitudinal photoperiodic variation. *Global Change Biol*. 2008 Nov;14(11):2516-22. (https://doi.org/10.1111/j.1365-2486.2008.01668.x)

33. Gow EA, Burke L, Winkler DW, Knight SM, Bradley DW, Clark RG, Bélisle M, Berzins LL, Blake T, Bridge ES, Dawson RD. 2019 A range-wide domino effect and resetting of the annual cycle in a migratory songbird. *Proc. R. Soc. B.* 286(1894):20181916. (https://doi.org/10.1098/rspb.2018.1916)

34. Cox AR, Robertson RJ, Rendell WB, Bonier F. 2020 Population decline in tree swallows
(*Tachycineta bicolor*) linked to climate change and inclement weather on the breeding ground. *Oecologia*.
1-0. (https://doi.org/10.1007/s00442-020-04618-8)

35. Møller AP, Flensted-Jensen E, Mardal W. 2009 Adjustment of the annual cycle to climatic change in a long-lived migratory bird species. *Curr Zool*. 55(2):92-101. (https://doi.org/10.1093/czoolo/55.2.92)

36. Tomotani BM, Gienapp P, Beersma DG, Visser ME. 2016 Climate change relaxes the time constraints for late-born offspring in a long-distance migrant. *Proc. R. Soc. B.* 283(1839):20161366. (https://doi.org/10.1098/rspb.2016.1366)

37. Berthold P. The control of migration in European warblers. 1988 InXIX Congressus Internationalis Ornithologici (pp. 215-249). *University of Ottawa Press*.

38. Gifford CE, Odum EP. 1965 Bioenergetics of lipid deposition in the bobolink, a trans-equatorial migrant. *Condor*. 67(5):383-403. (https://doi.org/10.2307/1365632)

39. Moore MC, Donham RS, Farner DS. 1982 Physiological preparation for autumnal migration in white-crowned sparrows. *Condor*. 84(4):410-9. (https://doi.org/10.2307/1367445)

40. Lindström Å, Daan S, Visser GH. 1994 The conflict between moult and migratory fat deposition: a photoperiodic experiment with bluethroats. Anim Behav. 48(5):1173-81.

(https://doi.org/10.1006/anbe.1994.1349)

41. Hall1 KS, Fransson T. 2001 Wing moult in relation to autumn migration in adult Common
Whitethroats Sylvia communis communis. *Ibis*. 143(3):580-6. (https://doi.org/10.1111/j.1474919X.2001.tb04885.x)

42. Bojarinova J, Babushkina O. 2015 Photoperiodic conditions affect the level of locomotory activity during autumn migration in the Long-tailed Tit (*Aegithalos c. caudatus*). *Auk*: Ornithological Advances. 132(2):370. (https://doi.org/10.1642/AUK-14-155.1)

43. Helm B, Van Doren BM, Hoffmann D, Hoffmann U. 2019 Evolutionary response to climate change in migratory pied flycatchers. *Curr Biol*. 29(21):3714-9. (https://doi.org/10.1016/j.cub.2019.08.072)

44. Wagner RH, Schug MD, Morton ES. 1996 Confidence of paternity, actual paternity and parental effort by purple martins. *Anim Behav*. 52(1):123-32. (https://doi.org/10.1006/anbe.1996.0158)

45. van Oppen MJ, Oliver JK, Putnam HM, Gates RD. 2015 Building coral reef resilience through assisted evolution. *P Natl A Sci.* 112(8):2307-13. (https://doi.org/10.1073/pnas.1422301112)

46. Fraser, KC. Migration in the Anthropocene. In Proppe, D. (ed.) Songbird Behavior: Implications for Conservation and Management in the Anthropocene. CRC Press, Boca Raton, Florida, USA. 2021

47. Rosenberg KV, Dokter AM, Blancher PJ, Sauer JR, Smith AC, Smith PA, Stanton JC, Panjabi A,
Helft L, Parr M, Marra PP. 2019 Decline of the North American avifauna. Science. 366(6461):120-4.
(10.1126/science.aaw1313)

Table 3.1: Top linear mixed-effects factors that explain variation in timing of fledge date and colony departure date of juveniles, as well as nesting duration and duration at the colony post-fledge. The variables we tested include the treatment, first egg date, and the number of nestmates.

 The global model of fledge date and nesting period also included individual fat score and weight at the time of radio-tagging.

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	W
	Fixed effects					
Fledge date	Treatment (control) *	-1.37±0.66	-2.66	-0.07		
	First egg date*	0.97 ± 0.07	0.82	1.12	453.1	0.33
	Nestmate numbers	0.44 ± 0.25	-0.05	0.95		
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	3.03	1.74	70.54		
	Fixed effects					
Duration in the nest (days)	Treatment (control) *	-1.05 ± 0.36	-1.76	-0.33		
	Nestmates numbers *	0.37 ± 0.15	0.06	0.68	409.2	0.57
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	0.65	0.80	34.20		
	Fixed effects					
	Treatment (control) *	-2.38 ± 0.88	-4.10	-0.65		
Colony departure date	First egg date*	0.90 ± 0.09	0.70	1.09	324.2	0.72
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	3.92	1.98	66.91		
Duration at the colony (days)	Fixed effects					
	First egg date	-0.12 ± 0.07	-0.27	0.02		
	Random effect	Variance	Std.Dev.	%Variance	344.5	0.38
	Cavity ID	1.72	1.31	36.69		



Figure 3.1: Duration in the nest for young in the treatment group that experienced extended day length as compared to young in the control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates mean. Whiskers extend to maximum and minimum values; outliers are indicated by filled points.



Figure 3.2: Duration at the colony for young in the treatment group that experienced extended day length as compared to young in the control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates mean. Whiskers extend to maximum and minimum values; outliers are indicated by filled points.



Figure 3.3: The influence of experimentally extended photoperiod on fledge and colony departure dates: a) shows the correlation between first egg date and fledge date; b) shows the correlation between first egg date and colony departure date. In figure 3 (a and b), each point represents individual birds that are independent and from different nest cavities.

Chapter 4: The Influence of Different Light Wavelengths of Anthropogenic Light at Night on Nestling Development and the Timing of Post-fledge Movements in a Migratory Songbird

Saeedeh Bani Assadi¹ and Kevin Charles Fraser¹

¹Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

Status: unpublished

Abstract:

Many different aspects of an animal's lifecycle such as its behavior, patterns of hormone activity, and internal clock time, can be affected by anthropogenic light at night (ALAN). Exposing an organism to ALAN during its early life could also have an impact on its development. Since photoperiod can trigger or schedule the migration timing of long-distance migratory birds, there is great potential for anthropogenic light to interact with photoperiod to affect timing. However, very little has been investigated regarding the impacts of ALAN on post-hatching development and migration timing. We investigated the impact of ALAN during nestling development in a long-distance migratory songbird to determine the potential impact on the timing of post-breeding movements in the wild. We experimentally manipulated the light by using programmable lighting, in the nest boxes of free-living nestlings of purple martin (Progne subis) in Manitoba, Canada. We exposed two groups of developing nestlings, from hatch to fledge date, to green or white LED lights (5 lux) during the night. We also included a control group that experienced natural, ambient light at night. We found that some adults abandoned their nests shortly after starting the experiment (4 of 15 nests in the white light treatment). For the nests that remained active, nestlings exposed to the white light treatment had higher weights (at day 20 or 22), later fledge dates (1.54 ± 0.37 , 95% CI 0.80–2.28), and later colony departure date $(2.84 \pm 1.00, 95\%$ CI 0.88–4.81), than young of the control group. Moreover, nestlings of both white and green light groups had longer nesting duration than nestlings of the control group. This study demonstrates the impact of ALAN on the development of post-breeding movement timing in nestlings of wild migratory birds. However, our results also indicate that green light may have less of an impact as compared to white light.

Keywords: Light pollution, Artificial Light At Night, phenotypic plasticity, postbreeding movements timing, ontogenetic effect

Introduction

With more than 50% of the global human population inhabiting cities (Nations, 2012), anthropogenic light at night (ALAN), or light pollution, has become one of the problems of urban sprawl for its impact on local environments (Rich and Longcore, 2005; Chepesiuk, 2009; Dominoni D. M., 2015). It is hypothesized that artificial light can be perceived as an extension of photoperiod in birds (Farner, 1964), where photoperiod can have a strong role in synchronizing internal clock time with seasonal rhythm (Berson et al., 2002). A study by Dominoni and Partecke (2015) on European blackbird (Turdus merula) showed that the impacts of light pollution on a bird's physiology and seasonal activities are comparable to the influence of longer photoperiods. Therefore, light pollution through its impact on internal clock time which controls many physiological processes (Foster and Kreitzmann, 2004), could impact different aspects of many animal's lifecycles, such as their natural behavior, and patterns of hormone activity (Rich and Longcore, 2005). Previous studies have revealed an impact of ALAN on the timing of activities such as the timing of singing of songbirds (e.g., Miller, 2006; Kempenaers et al., 2010; Da Silva et al., 2015), the timing of reproductive maturity (e.g, Dominoni and Partecke, 2015), and molt (Dominoni D. et al., 2013). For example, in European blackbird, urban light pollution (0.3 lux) resulted in an advance in their physiological phenotypes, where ALAN contributed to advances in the onset of reproductive development by 26 days (Dominoni D. et al., 2013). In general, and across the annual cycle, changes in timing as a result of exposure to ALAN may have negative fitness consequences, particularly if birds become mismatched with the timing of key resources needed for migration or breeding (Visser and Gienapp, 2019).

There is great potential for anthropogenic light to interact with natural photoperiod to influence perceived photoperiod and affect migration timing which could have fitness consequences (De Jong et al., 2015). However, there have been few studies on the impact of

LAN on the migration timing of animals, particularly birds. For example, Riley et al. (2013) found delays in the dispersal of Atlantic salmon (*Salmo salar*) fry who were exposed to ALAN. Also, Smith et al. (2021) showed that adult purple martins (*Progne subis*) who experienced ALAN for more than 10 nights, initiated spring migration 8 days earlier than others who experienced natural darkness. This advance in timing was not compensated for during migration and birds experiencing ALAN that had left earlier also arrived at their breeding grounds 8 days earlier, suggesting the potential for mismatch between bird timing and the availability of resources in early spring. Further studies with other species and at different times of year are now required to further improve our understanding of the impact of ALAN on migration timing. Further, to our knowledge, there has been no study that has investigated the ontogenetic effects of light pollution on movement timing of long-distance migratory songbirds.

In this study, we used purple martin which is a gregarious long-distance Nearctic-Neotropical migratory songbird that journeys 10–20,000 km annually between breeding sites across eastern North American and overwintering locations in South America (Fraser et al., 2012; Neufeld et al., 2021). Considering the potential interaction between light pollution and photoperiod that may impact timing, the objectives of this study were to determine the ontogenetic effects of ALAN during nesting on the subsequent development of post-breeding movement timing in young birds. For this purpose, we experimentally exposed free-living nestlings to artificial light. To also examine whether different spectra of light have different impacts, we used both white and green lights (long and near short wavelength) in our experiment. The different impacts of green versus white light in a lab study on daily rhythms of blue tit (*Cyanistes caeruleus*) (De Jong et al., 2017), provided context for our investigation of the impacts of different wavelengths on the timing of post-breeding movements in the wild.
We subsequently tracked individuals as they fledged using an automated telemetry system (Taylor et al., 2017) to allow us to determine the responses of their timing to the simulated light pollution. We predicted that the light treatments would have some impacts on physiology as well as on timing, which could be measured through their weight and departure timing. We predicted that nestlings of the experimental groups would have later fledge dates and post-fledge movement timing in comparison with the control group. As songbirds may perceive light pollution as similar to longer day length (Dominoni and Partecke, 2015), we expected that birds in the experimental groups would have later timing of post-breeding movements. This is because a longer day length at the study latitude would simulate an earlier calendar date, as days get shorter through the nesting period. It was also expected that development and weight gain in birds experiencing the light treatments would be slower than those in the control group, as they may be more active under constant light at night. Regarding the study results of De Jong et al. (2017) that both white and green light at an intensity of 5 lux had the same influence on daily rhythms of blue tit (*C. caeruleus*), we predicted the influence of both spectra of lights would be the same.

Materials and Methods

This study was conducted at two purple martin colonies in southern Manitoba, located just south of Winnipeg (49.7348° N, 97.1301° W) and at Altona (49.126748°, –97.570463°). Purple martins are dependent upon human-supplied houses for nesting, which have multiple nest boxes per housing unit (Brown et al., 2021). We used five purple martin houses for our study. Four of these houses had a total of 14 nest boxes each and the fifth house had 32 nest boxes. We used 33 nest boxes from the location south of Winnipeg and 14 nest boxes from the location at Altona in our experiment. To investigate the impact of ALAN on the timing of postbreeding movements of juveniles, the light within purple martin nest boxes was manipulated during dark hours. Light-emitting diodes (LED) in two colors (white and green) were attached

to the ceiling of each nest box before nestlings hatched. Light emitted by the LEDs was directed downward toward the nests (Raap et al., 2016b, a). To control the time that lights turned on and off, LEDs were connected to an Arduino unit (a circuit board that can be programmed with associated software to set the light schedules) and a real-time clock mounted on a circuitboard. The LEDs were programmed to turn on at sunset, stay on during the entire night, and turn off at sunrise of the next day (Supplementary Figure 4.1). The experimental period began 3–4 days before the hatch date of each nest and continued until the fledge date. The nest boxes were divided into three different experimental groups: controls (no light, dark), treatment group 1 (white light), and treatment group 2 (green light). Light intensity for both treatment groups was set at 5 lux. The dim, 5 lux was chosen to align with De Jong et al. (2017), where in the lab there was no measured difference in the impact between green and white light at this intensity. Therefore, this provided us with the opportunity to compare results and investigate impacts on the timing of post-breeding movements on a migratory species in the wild. In total, each of the control and green light groups included 16 nest boxes each, and the white light group included 15 nest boxes. To determine the fledge date and colony departure date of young, we used the Motus Wildlife Tracking System, 1 which is a continent-wide automated radio-telemetry array of receiver stations (Taylor et al., 2017). At each of our research colonies, we installed a Motus receiver, within 8–70 m of the cavities. We randomly selected individuals (3–5 where available and of adequate weight for tagging) from each nest box and equipped them with individually coded radio nanotags (NTQB2-3-2 Lotek Inc.) (0.62 g, $12 \times 6 \times 5$ mm in length, width, and height, respectively) using a leg-loop harness design (Rappole and Tipton, 1991; Streby et al., 2015) made of black elastic sewing thread (~ 0.5 mm). Tag deployment was conducted when nestlings were near fledging at the age of 20–22 days (post-hatch). At the time of tagging, the weight of each nestling was recorded by using a digital scale with a resolution of 0.01 g. The weight of a tag and harness was less than 3% of the weight of the juveniles (~54.27 grams)

(the average weight of nestlings). Each nanotag emitted a signal every 29 s and had a battery life of approximately 367 days.2 After turning on the lights, 4 of the nests in the white light group were abandoned. In total, 61, 55, and 49 tags were deployed on nestlings from nests that remained active in each of the control, green and white light groups, respectively.

The fledge date and colony departure date were determined by using a combination of variation in signal strength of each nanotag and complementary nest checks every other day. The date of fledging was determined when after a constant signal fluctuation (indicating the tagged bird is in the nest at a constant distance from the receiver), we observed a great fluctuation in signal strength which indicates fledging from the cavity. After this great fluctuation of signal strength, we observed repetition of this pattern which shows the fledged bird was rapidly changing position in relation to the receiver. Colony departure date was determined according to the fading signals of the tagged birds and the last detections of the tagged bird at the colony site (Supplementary Figure 4.2). Biologically unrealistic false positive detections were omitted (e.g., from distant receivers pre-fledge). Where tag signals ceased (e.g., owing to tag malfunction, predation, or another unknown cause) or in cases where a constant signal indicated a tag had fallen off or a bird had died, data were removed from further analysis.

All data collection procedures and experiments were conducted in accordance with the guidelines of the University of Manitoba's Animal Care Committee who have approved this project [Animal Care Protocol Number F18-031/1(AC11388)].

Data Analysis

For examining the effect of ALAN on the timing of fledge date of juveniles and their nesting duration linear mixed-effects models (LMMs) were fit by REML using the "lme4" package (Bates et al., 2014). The variables of weight (gram), first egg date, treatment (green or white light), and the number of nestmates were assigned as fixed effects and cavity ID and colony as the random effects. As only three nests had a second-year parent and all other adults

were after-second year, the age of parents was not included in the analyses. To investigate the impact of ALAN on the duration (days) at the colony (hatch to departure) and colony departure date, we used LMMs with the same variables of first egg date, treatment (green or white light), and the number of nestmates as fixed and cavity ID and colony as random effects, except for the weight of the young as this was not possible to measure after their fledging. Preliminary investigation using likelihood ratio tests revealed that the random effect of the colony was not significant in the models (fledge date: $\chi^2 = 0$, P = 1, duration in the nest: $\chi^2 = 0$, P = 1; colony departure date: $\chi 2 = 0$, P = 0.99; duration at the colony: $\chi 2 = 0$, P = 1), and the models only converged with random effect of cavity ID. Therefore, to meet model parsimony, the factor of colony was omitted from further analysis. The distribution of residuals of each model was assessed to meet the assumption of normality and equality of variance (Zuur et al., 2010). The collinearity of variables in each model was assessed, which was less than 2 for all variables. To run the possible candidate models from the full model, Akaike Information Criteria corrected for small sample size (AICc) was used (Burnham and Anderson, 2002) through the package "MuMIn" (Barton, 2019). The best model among the competitive models with $\Delta AICc$ < 2 was selected (Supplementary Tables 4.1–4.3) according to the highest value of the marginal R2 (variance explained by the fixed effects) and the conditional R2 (variance explained by the whole model) (Barton, 2019). ANCOVA was used to test the interaction of treatments (categorical variable) with first egg date (continuous variable) for both dependent factors, fledge date and colony departure date (McDonald, 2014). One-way ANOVA was used to investigate whether the mean weight of juveniles is different among different groups (white light, green light, and control), and where applicable, to explore the differences among weight means of three groups, the package "lmerTest" (Kuznetsova et al., 2017) was used to run Tukey HSD tests for post hoc analyses.

The survival rate of young of each nest box was calculated by dividing the number of fledged young by the number of hatched nestlings of each nest box. Due to the non-normal distribution of data, a Kruskal-Wallis test was used to compare the survivability rate among three groups (white light, green light, and control groups). All analyses were conducted in R version 3.6.3 (R Core Team, 2020).

Results

We tracked the fledge date of 61, 47, and 46 individuals of the control, green light and white light groups, respectively. Among these tagged nestlings, we were able to track the colony departure dates of 45 individuals from the control group, and 20 and 33 individuals of the green and white light groups, respectively.

Nesting Duration and Fledge Date

The average nesting duration of the control, green light and white light groups were (mean \pm SEM) 28.16 \pm 0.15, 29 \pm 0.15, and 30.11 \pm 0.23 days, respectively. Nestlings exposed to green light and white light spent (estimate \pm SE) 0.78 \pm 0.32 (95% CI 0.14–1.41) and (estimate \pm SE) 1.67 \pm 0.34 (95% CI 1.00–2.34) days, respectively, longer in the nest than those nestlings who experienced natural darkness during the night (Table 1). Moreover, one additional nestmate and a 1-gram increase in weight resulted in nesting duration that was longer by (estimate \pm SE) 0.30 \pm 0.13 (95% CI 0.03–0.57) and (estimate \pm SE) 0.04 \pm 0.02 (95% CI 0.00–0.08) days, respectively (Table 1).

Overall, fledge dates ranged from 12 July to 3 August. Average fledge dates of control, green light and white light groups were dates 21 July (mean \pm SEM) (203 \pm 0. 59), 24 July (206 \pm 0.65), and 25 July (207.39 \pm 0.52), respectively. Nestlings exposed to white light fledged (estimate \pm SE) 1.54 \pm 0.37 (95% CI 0.80–2.28) days later than nestlings of the control group (Table 1). There was not a significant difference between the fledge dates of nestlings of the green light group and the control group. Moreover, first egg dates that were one day later

resulted in nestling fledge dates that were (estimate \pm SE) 1.01 \pm 0.03 (95% CI 0.93–1.08) days (Figure 1A and Table 1) later. One more nestmates in a cavity delayed fledge date about (estimate \pm SE) 0.31 \pm 0.14 (95% CI 0.02–0.60) days (Table 1). The results of ANCOVA showed the effect of first egg date on the fledge date is independent of the treatments or different colors of ALAN and it is assumed the slopes are similar (Figure 1A and Supplementary Table 4.4).

Duration at the Colony and Colony Departure Date

The best model of duration at the colony did not include any of the fixed effects as influential factors (Table 1). Nestlings who experienced white light at night departed the colony (estimate \pm SE) 2.84 \pm 1.00 (95% CI 0.88–4.81) days later than those of the non-treatment group (Table 1). The results of ANCOVA showed the effect of first egg date on the colony departure date was similar among the different treatments of ALAN (Figure 1B and Supplementary Table 4.4). Moreover, one day delay in first egg date resulted in nestlings departing the colony (estimate \pm SE) 0.94 \pm 0.12 (95% CI 0.71–1.18) days later (Figure 1B and Table 1).

Weight

The average weight of nestlings of the control, green light and white light groups at day 20–22 were (mean \pm SEM) 53.50 \pm 0.63, 53.85 \pm 0.65, and 56.10 \pm 0.92, respectively (Figure 2). The differences of the mean weight of nestlings among groups were significant (DF = 2, F = 3.27, p = 0.04). The Tukey post hoc test showed the mean weight of nestlings of the white light group was significantly more than those in the control group (estimate \pm SE) (2.6 \pm 1.06, *p* = 0.03) when they were 20–22 days old. There was no significant difference between the weight of nestlings of the green light group and the weight of nestlings of both the control and white light groups.

Survivability Rate

Three of 16 control group nests and 4/16 green light group nests did not fledge completely with 6 and 9 nestlings lost in each group, respectively. In the white light group, 4 out of 15 nests were abandoned at the beginning of the experiment and 2 out of the remaining 11 nests did not fledge completely. This resulted in a total of 10 nestlings lost from the white light group. There was not a significant difference in the nest survivability rate between the treatment groups, (white and green light groups) and the control group, nor between the two treatment groups ($\chi^2 = 0.17$, p = 0.91). The actual numbers of fledged young were 79, 74 and 49 from the control, green light, and white light groups, respectively. Nestlings that disappeared between nest checks were not found and may have been taken by avian predators (Brown et al., 2021), as predator guards would prevent terrestrial predators from accessing the nest boxes.

Discussion

In this study, we demonstrate for the first time that ALAN impacts the timing of the post-breeding movements of juveniles of a long-distance migratory songbird. We examined the ALAN impacts of different spectra of light (white and green lights at 5 lux) on the duration of nesting and timing of fledge and post-breeding movements. We found that the effects of different spectra differed for the post-breeding movement timing of juvenile purple martins. Our results reveal that exposing nestlings to white light with an intensity of 5 lux during the night, resulted in later fledging and colony departure as compared to nestlings who experienced either green light or natural darkness. Thus, our data reveal important effects of ALAN on timing but that these differ by spectra of light. We found that green light with an intensity of 5 lux during the not influence the timing of the post-breeding movement of young purple martins. However, nestlings exposed to either white or green light had a longer nesting duration than nestlings of the control group (that experienced natural darkness).

Evidence to date suggests that ALAN can influence the perception of photoperiod by birds (De Jong et al., 2015), influencing their internal clock time and consequently impacting biological functions and fitness (Farner, 1964; Dominoni D. M., 2015). Previous studies which investigated the impact of light pollution on circadian rhythms of songbirds revealed that it can cause a phase shift in their circadian rhythm (Gaston et al., 2013) and advance or delay the onset and offset of their daily activities such as singing (e.g., Kempenaers et al., 2010; Da Silva et al., 2014), foraging activity (Russ et al., 2015), and timing of reproduction (Kempenaers et al., 2010; De Jong et al., 2015). Moreover, egg-laying date is influenced by day length as one of the important zeitgebers (Lambrechts et al., 1997; Da Silva et al., 2015). De Jong et al. (2015) found when the temperature was low in spring in comparison with a warmer spring, light pollution at night was perceived as a longer photoperiod by great tit (Parus major) which changed the onset of egg-laying.

In addition to changes in the timing of nesting activities due to ALAN, a study by Smith et al. (2021), demonstrated the advancement of spring migration departure of adult purple martins who experienced light pollution during their overwintering period. One of the reasons posed for this advance was that ALAN led to a perception of a longer day length which mimicked a later calendar date, causing earlier development of reproductive organs (Smith et al., 2021). In this study, we showed an ontogenetic effect of ALAN at breeding sites on nestlings, which resulted in later post-breeding movements. This delay could be due to the impact of ALAN in the nest on the growth rate of nestlings, where birds exposed to ALAN are heavier leading to a delay in timing. An earlier study that compared the weights of nestlings exposed to white light (3 lux) with controls found similar results where ALAN-exposed nestlings gained more weight than those in the control group (Gagné, 2019). Previous studies revealed that a typical pattern of weight gain and loss in nestling purple martins is for them to lose weight as they approach fledge date, possibly to achieve a weight more suited to fledging and first flights (Allen and Nice, 1952; Dellinger and Rogillio, 1991; Gagné, 2019). White light may influence this natural pattern, changing the metabolism or the proportion of rest and active periods of the nestlings and increasing begging for food (Raap et al., 2016c) which could consequently prolong the duration of feeding by adults. This aligns with the results of a study by Titulaer et al. (2012) that showed an increase in the feeding rate of great tit females when nestlings were exposed to ALAN (10 lux) while they were between 9 and 16 days old. However, exposing free-living great tit nestlings to artificial light at night (3 lux), even for two nights, during their development led to substantial impacts on their physiological condition (Raap et al., 2016b) and increased their activity levels which resulted in the nestlings having no weight gain for the two nights of the experimental treatment (Raap et al., 2016a). Our results align with these earlier studies as we found that the weight of nestlings of the white light group was significantly more than the nestlings of the control group. We infer that given that nestling martins tend to lose weight just before fledging, that the higher weight of white light exposed nestlings may have led to the later fledge dates that we observed. However, we found no significant difference in weight between individuals in the green group and the other two groups.

While the ontogenetic effect of photoperiod on circadian rhythms of mammals has been demonstrated (e.g., Ciarleglio et al., 2011), there is scant information about the ontogenetic effects of daylength on avian migration timing (Knudsen et al., 2011). This is particularly so for the potential ontogenetic effects of light pollution. The ontogenetic effect of hatch date on spring migration timing of pied flycatcher and spring arrival time of Arctic terns (Sterna paradisea) has been suggested by Møller et al. (2009) and Both (2010), respectively. Moreover, in a previous study, we found phenotypic plasticity of post-breeding movement timing of young purple martin to an experimentally extended day length during their nesting (Bani Assadi and Fraser, 2021). In our study, the longer nesting period and later fledge date of nestlings exposed to the white light treatment, could potentially indicate a plasticity of post-breeding movements timing of nestlings to the ontogenetic effects of light during the nesting

period, with carry-over effects on the timing of their colony departure date. How long these timing effects may last across the rest of the annual cycle, and how they trade-off these delays in timing in the next stage of their life cycle, are important areas of investigation for future research. Delays in timing may be compensated for during fall migration, or during the winter. In wood thrushes, fall migration may mitigate the carry-over effect of late-breeding timing (Stutchbury et al., 2011; Catry et al., 2013) and Gow et al. (2019) found that stationary periods during the non-breeding season in tree swallows may act as a timing reset period, removing carry-over effects on timing from the breeding season and fall migration. However, some impacts on timing may have longer-lasting effects, particularly on juvenile birds. Ouwehand et al. (2017) showed that the carry-over effect of an experimentally delayed hatch for juvenile pied flycatchers extended to spring arrival date back at breeding sites in the following year. While we did not track the timing of our experimental birds to the subsequent spring, based upon the results of Ouwehand et al. (2017), we would expect to observe a carry-over effect of ALAN on their spring arrival date.

Similar to a study of De Jong et al. (2015), which showed a lack of impact of ALAN on the survivability rate of great tits nestlings, we did not detect a difference in the survivability rate (fledging) of the young among our three groups (white light, green light, and controls). However, previous studies have revealed some non-lethal but negative impacts of ALAN via increases in stress hormones (Ouyang et al., 2015) and decreases in melatonin levels (Dominoni D. M. et al., 2013). For example, a field study by Raap et al. (2016b) showed that exposing great tit nestlings to ALAN (0.3 lux) for two nights when they were 13 days old caused a deterioration of their immunity and health condition via a decrease in melatonin and an increase in oxidative stress and stress hormones. Melatonin is secreted by the pineal gland at night and plays an important role in maintaining the circadian rhythm (Raap et al., 2015). A disruption in circadian rhythm can impact several immune responses (Arjona et al., 2012). In

our study, despite the lack of influence of two spectra of lights (white and green) on the survivability rate of young, we cannot rule out more subtle effects on their health condition and how this may influence migration. This would therefore be an important future research avenue, as the impact of ALAN on complex neuroendocrine functions, and how this may differ as they traverse different environments, is unknown (Haldar and Singh, 2001).

How different wavelengths of light may impact the behavior and physiology of birds have yielded mixed results across studies, species and time of year. For example, in a field study, Ouyang et al. (2015) showed that the concentration of stress hormone in great tits was greater when nests were closer to white lights in comparison with individuals with nests near green lights (8.2 \pm 0.3 lux). Moreover, wavelengths around the blue spectrum have been demonstrated to be more influential on the reproductive physiology of birds (Dominoni D. M., 2015) and laying date (De Jong et al., 2015) than other spectra of light. In our field study, white light of higher intensity (5 lux) was influential on post-breeding movement timing of wild young purple martin, while green light with the same intensity did not have any impact. In contrast with our findings, a laboratory study by De Jong et al. (2017) revealed that at low intensity (0.5 and 1.5 lux), the daily rhythm of blue tits (C. caeruleus) was more disturbed under white and red lights than green light. However, they found that at a higher range of intensities (5 lux), both white and green lights had the same negative impact on the circadian rhythms of a blue tit. In another study De Jong et al. (2015), found that the lay date of great tit was influenced by white and green lights at night (8.2 \pm 0.3 lux out of the nest, but 0.05 lux in the nests), however, pied flycatchers' lay date was not impacted by ALAN (De Jong et al., 2015). In contrast with this result, a study by Poot et al. (2008), showed an influence of the long-wavelength spectrum (red and white lights) where it caused nocturnal migratory birds to be disoriented during flight. In general, the greater influence of white light at low intensity across studies could be due to its greater penetration of the skull as compared to green light, where it may have a corresponding impact on photoreceptors (Hartwig and van Veen, 1979).

In addition to the impacts of our experimental treatments, we found that other factors also impacted timing as expected. For example, we found that first egg date was an influential factor in the timing of fledge date and colony departure date. The number of nestmates also impacted fledge date, which aligns with the results of Wagner et al. (1996), where an increase in the number of nestlings of purple martins increases competition for food and therefore they require more time to reach the optimal body condition for fledging. Among the zeitgebers that may influence timing, photoperiod is expected to play the largest role in synchronizing internal clock time (Gwinner, 1996; Åkesson and Helm, 2020). Therefore, it was expected that longer day lengths experienced by birds that hatched earlier would induce them to have earlier fledge dates (Coppack and Pulido, 2004). However, our investigation did not show any interactions between the experimental light treatments and first egg date.

Conclusion

This study demonstrated the ontogenetic effects of white ALAN during the nesting period on the timing of post-breeding movements of juvenile birds. However, we did not find any significant difference in the timing of post-breeding movements for birds that were exposed to green ALAN as compared to controls. The potential for negative carryover effects on other stages of the annual cycle (Norevik et al., 2017) or whether the carryover effects of light pollution on migration timing are compensated for during migration or during the overwintering period (Senner et al., 2014; Briedis et al., 2018; Gow et al., 2019) requires further investigation. The negative impact of white light on the circadian or circannual rhythm of young migratory songbirds that we demonstrate, and the lack of influence of green light leads to the recommendation of using a shorter wavelength (green light) for illuminating places that are close to breeding sites.

Acknowledgments

We thank Gail Davoren, Colin Garroway, and Saman Muthukumarana for helpful comments on earlier drafts of this manuscript. We thank Leanne Neufeld for her assistance with fieldwork. We especially thank purple martin colony managers Alan Enns, Paul and Maxine Clifton for their patience and assistance with this research.

Funding

Funding and other supports were provided by John R. Evans Leaders Fund, Canadian Foundation for Innovation, Research Manitoba, the Natural Sciences and Engineering Research Council's Discovery Grant Program, the Connie Holland Bird Study Fund, and the University of Manitoba.

References

- Åkesson, S., and Helm, B. (2020). Endogenous programs and flexibility in bird migration. *Front*. *Ecol. Evol.* 8:78. doi: 10.3389/fevo.2020.00078.
- Allen, R. W., and Nice, M. M. (1952). A study of the breeding biology of the Purple Martin (*Progne subis*). *Am. Mid. Nat.* 47, 606–665. doi: 10.2307/2422034
- Arjona, A., Silver, A. C., Walker, W. E., and Fikrig, E. (2012). Immunity's fourth dimension: approaching the circadian–immune connection. *Trends Immunol*. 33, 607–612. doi: 10.1016/j.it.2012.08.007.
- Bani Assadi, S., and Fraser, K. C. (2021). Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird. *Proc. R. Soc. B.* 288:20211474. doi: 10.1098/rspb.2021.1474
- Barton, K. (2019). MuMIn: Multi-Model Inference, R Package Version 1.42.1.
- Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv* [Preprint].
- Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073. doi: 10.1126/science.1067262.
- Both, C. (2010). Flexibility oftiming of avian migration to climate change masked by environmental constraints en route. *Curr. Biol.* 20, 243–248. doi: 10.1016/j.cub.2009.11.074.
- Briedis, M., Krist, M., Král, M., Voigt, C. C., and Adamík, P. (2018). Linking events throughout the annual cycle in a migratory bird-non-breeding period buffers accumulation of carry-over effects. *Behav. Ecol. Sociobiol.* 72:33.
- Brown, C. R., Airola, D. A., and Tarof, S. (2021). Purple Martin (Progne subis), version 2.0. In Birds of the World, ed. P. G. Rodewald (Ithaca, NY: The Cornell Lab of Ornithology).
- Burnham, K. P., and Anderson, D. R. (2002). A Practical Information-Theoretic Approach. Model Selection And Multimodel Inference. Vol. 10, New York, NY: Springer.
- Catry, P., Dias, M. P., Phillips, R. A., and Granadeiro, J. P. (2013). Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology* 94, 1230–1235. doi: 10.1890/12-2177.1
- Chepesiuk, R. (2009). Missing the dark: health effects of light pollution. *Environ. Health Perspect.* 117, A20–A27.
- Ciarleglio, C. M., Axley, J. C., Strauss, B. R., Gamble, K. L., and McMahon, D. G. (2011). Perinatal photoperiod imprints the clock. *Nat. Neurosci.* 14, 25–27.

- Coppack, T., and Pulido, F. (2004). Photoperiodic response and the adaptability of avian life cycles to environmental change. *Adv. Ecol. Res.* 35, 131–150. doi: 10.1016/s0065-2504(04)35007-5
- Da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., and Kempenaers, B. (2014). Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behav. Ecol.* 25, 1037–1047. doi: 10.1093/beheco/aru103
- Da Silva, A., Valcu, M., and Kempenaers, B. (2015). Light pollution alters the phenology of dawn and dusk singing in common European songbirds. *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140126. doi: 10.1098/rstb.2014.0126.
- De Jong, M., Caro, S. P., Gienapp, P., Spoelstra, K., and Visser, M. E. (2017). Early birds by light at night: effects of light color and intensity on daily activity patterns in blue tits. *J. Biol. Rhythms* 32, 323–333. doi: 10.1177/0748730417719168
- De Jong, M., Ouyang, J. Q., Da Silva, A., van Grunsven, R. H., Kempenaers, B., Visser, M. E., et al. (2015). Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species. *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140128. doi: 10.1098/rstb.2014.0128
- Dellinger, T. B., and Rogillio, C. (1991). The graphic development of baby Purple Martins. *Purple Martin Update* 3, 6–11.
- Dominoni, D. M. (2015). The effects of light pollution on biological rhythms of birds: an integrated, mechanistic perspective. *J. Ornithol.* 156, 409–418. doi: 10.1007/s10336-015-1196-3.
- Dominoni, D. M., and Partecke, J. (2015). Does light pollution alter daylength? A test using light loggers on free-ranging European blackbirds (*Turdus merula*). *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140118. doi: 10.1098/rstb.2014.0118
- Dominoni, D., Quetting, M., and Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. B Biol. Sci.* 280:20123017. doi: 10.1098/rspb.2012.3017
- Dominoni, D. M., Goymann, W., Helm, B., and Partecke, J. (2013). Urban-like night illumination reduces melatonin release in European blackbirds (*Turdus merula*): implications of city life for biological time-keeping of songbirds. *Front. Zool.* 10:1–11. doi: 10.1186/1742-9994-10-60
- Farner, D. S. (1964). The photoperiodic control of reproductive cycles in birds. *Am. Sci.* 52, 137–156.
- Fraser, K. C., Stutchbury, B. J., Silverio, C., Kramer, P. M., Barrow, J., Newstead, D., et al. (2012). Continent-wide tracking to determine migratory connectivity and tropical habitat associations

of a declining aerial insectivore. *Proc. R. Soc. B Biol. Sci.* 279, 4901–4906. doi: 10.1098/rspb.2012.2207

- Foster, R. G., and Kreitzmann, L. (2004). *Rhythms of Life: The Biological Clocks That Control the Daily Lives of Every Living Thing*. New Haven, NH: Yale University Press.
- Gagné, M. (2019). The Effect Of Artificial Light At Night (Alan) On The Growth And Migration Timing Of Purple Martins (Progne Subis). Unpublished honours Thesis University of Manitoba, Winnipeg.
- Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036
- Gow, E. A., Burke, L., Winkler, D. W., Knight, S. M., Bradley, D. W., Clark, R. G., et al. (2019). A range-wide domino effect and resetting of the annual cycle in a migratory songbird. *Proc. R. Soc. B Biol. Sci.* 286:20181916. doi: 10.1098/rspb.2018.1916
- Gwinner, E. (1996). Circadian and circannual programmes in avian migration. *J. Exp. Biol.* 199, 39–48. doi: 10.1242/jeb.199.1.39
- Haldar, C., and Singh, S. S. (2001). Seasonal changes in melatonin and immunological adaptations in birds. J. Endocrinol. Reprod. 5, 13–24.
- Hartwig, H. G., and van Veen, T. (1979). Spectral characteristics of visible radiation penetrating into the brain and stimulating extraretinal photoreceptors. J. Comp. Physiol. 130, 277–282. doi: 10.1007/bf00614615
- Kempenaers, B., Borgström, P., Loës, P., Schlicht, E., and Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Curr. Biol.* 20, 1735– 1739. doi: 10.1016/j.cub.2010.08.028
- Knudsen, E., Lindén, A., Both, C., Jonzén, N., Pulido, F., Saino, N., et al. (2011). Challenging claims in the study of migratory birds and climate change. *Biol. Rev.* 86, 928–946.
- Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). ImerTest Package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. doi: 10.18637/jss.v082.i13
- Lambrechts, M. M., Blondel, J., Maistre, M., and Perret, P. (1997). A single response mechanism is responsible for evolutionary adaptive variation in a bird's laying date. *PNAS* 94, 5153–5155. doi: 10.1073/pnas.94.10.5153
- McDonald, J. H. (2014). *Handbook of Biological Statistics*, 3rd Edn. Baltimore, MD: Sparky House Publishing.
- Miller, M. W. (2006). Apparent effects of light pollution on singing behavior of American robins. *Condor* 108, 130–139. doi: 10.1650/0010-5422(2006)108[0130:aeolpo]2.0.co;2

- Møller, A. P., Flensted-Jensen, E., and Mardal, W. (2009). Adjustment of the annual cycle to climatic change in a long-lived migratory bird species. *Curr. Zool.* 55, 92–101. doi: 10.1093/czoolo/55.2.92
- Nations, U. (2012). World Urbanization Prospects: The 2014 Revision, CD-ROM Edn. New York, NY: DESA.
- Neufeld, L. R., Muthukumarana, S., Fischer, J. D., Ray, J. D., Siegrist, J., and Fraser, K. C. (2021).
 Breeding latitude is associated with the timing of nesting and migration around the annual calendar among Purple Martin (*Progne subis*) populations. *J. Ornithol.* 18, 1–16.
- Norevik, G., Åkesson, S., and Hedenström, A. (2017). Migration strategies and annual space-use in an Afro-Palaearctic aerial insectivore–the European nightjar Caprimulgus europaeus. J. Avian Biol. 48, 738–747.
- Ouwehand, J., Burger, C., and Both, C. (2017). Shifts in hatch dates do not provide pied flycatchers with a rapid ontogenetic route to adjust offspring time schedules to climate change. *Funct. Ecol.* 31, 2087–2097. doi: 10.1111/1365-2435.12940.
- Ouyang, J. Q., de Jong, M., Hau, M., Visser, M. E., van Grunsven, R. H., and Spoelstra, K. (2015). Stressful colours: corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination. *Biol. Lett.* 11:20150517. doi: 10.1098/rsbl.2015.0517
- Poot, H., Ens, B. J., de Vries, H., Donners, M. A., Wernand, M. R., and Marquenie, J. M. (2008). Green light for nocturnally migrating birds. *Ecol. Soc.* 13:47.
- R Core Team (2020). *R: A Language And Environment For Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria.
- Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., et al. (2016a). Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study. *Sci. Rep.* 6, 1–8.
- Raap, T., Casasole, G., Pinxten, R., and Eens, M. (2016b). Early life exposure to artificial light at night affects the physiological condition: an experimental study on the ecophysiology of freeliving nestling songbirds. *Environ. Pollut.* 218, 909–914. doi: 10.1016/j.envpol.2016.08.024
- Raap, T., Pinxten, R., and Eens, M. (2016c). Artificial light at night disrupts sleep in female great tits (*Parus major*) during the nestling period, and is followed by a sleep rebound. *Environ. Pollut.* 215, 125–134. doi: 10.1016/j.envpol.2016.04.100
- Raap, T., Pinxten, R., and Eens, M. (2015). Light pollution disrupts sleep in free-living animals. Sci. Rep. 5, 1–8.

- Rappole, J. H., and Tipton, A. R. (1991). New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). J. Field Ornithol. 62, 335–337.
- Rich, C., and Longcore, T. (2005). *Ecological Consequences of Artificial Night Lighting*.Washington, DC: Island Press.
- Riley, W. D., Davison, P. I., Maxwell, D. L., and Bendall, B. (2013). Street lighting delays and disrupts the dispersal of Atlantic salmon (*Salmo salar*) fry. *Biol. Conserv.* 158, 140–146. doi: 10.1016/j.biocon.2012.09.022
- Russ, A., Reitemeier, S., Weissmann, A., Gottschalk, J., Einspanier, A., and Klenke, R. (2015).
 Seasonal and urban effects on the endocrinology of a wild passerine. *Ecol. Evol.* 5, 5698–5710. doi: 10.1002/ece3.1820
- Senner, N. R., Hochachka, W. M., Fox, J. W., and Afanasyev, V. (2014). An exception to the rule: carry-over effects do not accumulate in a long-distance migratory bird. *PLoS One* 9:e86588. doi: 10.1371/journal.pone.0086588
- Smith, R. A., Gagné, M., and Fraser, K. C. (2021). Pre-migration artificial light at night advances the spring migration timing of a trans-hemispheric migratory songbird. *Environ. Pollut.* 269:116136. doi: 10.1016/j.envpol.2020.116136
- Streby, H. M., McAllister, T. L., Peterson, S. M., Kramer, G. R., Lehman, J. A., and Andersen, D. E. (2015). Minimizing marker mass and handling time when attaching radio-transmitters and geolocators to small songbirds. *Condor* 117, 249–255. doi: 10.1650/condor-14-182.1
- Stutchbury, B. J., Gow, E. A., Done, T., MacPherson, M., Fox, J. W., and Afanasyev, V. (2011). Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proc. R. Soc. B Biol. Sci.* 278, 131–137. doi: 10.1098/rspb.2010.1220
- Taylor, P., Crewe, T., Mackenzie, S., Lepage, D., Aubry, Y., Crysler, Z., et al. (2017). The motus wildlife tracking system: a collaborative research network to enhance the understanding of wildlife movement. *Avian Conserv. Ecol.* 18:8.
- Titulaer, M., Spoelstra, K., Lange, C. Y. M. J. G., and Visser, M. E. (2012). Activity patterns during food provisioning are affected by artificial light in free living great tits (*Parus Major*). *PLoS One* 7, 5–8. doi: 10.1371/journal.pone.0037377
- Visser, M. E., and Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nat. Ecol. Evol.* 3, 879–885. doi: 10.1038/s41559-019-0880-8

- Wagner, R. H., Schug, M. D., and Morton, E. S. (1996). Condition-dependent control of paternity by female purple martins: implications for coloniality. *Behav. Ecol. Sociobiol.* 38, 379–389. doi: 10.1007/s002650050255
- Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi: 10.1111/j.2041-210x.2009.00001.x

Table 4.1: The best models of the linear mixed-effects analysis of effects of ALAN,weight, first egg date, nestmate numbers on nesting period, fledge date, and effects of ALAN,first egg date, nestmate numbers on duration of staying at the colony and colony departure date.The cavity ID is considered as a random effect.

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
	Fixed effects					
Fledge date	Treatment (green light)	0.54 ± 0.36	-0.15	1.25		
	Treatment (white light) *	1.54 ± 0.37	0.80	2.28	544.4	0.90/0.92
	First egg date*	1.01 ± 0.03	0.93	1.08		
	Nestmate numbers*	0.31 ± 0.14	0.80	2.28		
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	0.48	0.69	24.61		
	Fixed effects					
Duration in the nest (days)	Treatment (green light) *	0.78 ± 0.32	0.14	1.41		
	Treatment (white light) *	1.67 ± 0.34	1.00	2.35	529.5	0.28/0.43
	Weight*	0.04 ± 0.02	0.00	0.08		
	Nestmate numbers*	0.30 ± 0.13	0.03	0.57		
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	0.37	0.61	21.14		
Colony departure date	Fixed effects					
	Treatment (green light)	0.54 ± 1.07	-1.57	2.65		
	Treatment (white light) *	2.84 ± 1.00	0.88	4.81	504.2	0.57/0.71
	First egg date*	0.94 ± 0.12	0.70	1.18		
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	3.21	1.79	32.44		
Duration at the colony (days)	Fixed effects					
	Null				472.4	0/0.34
	Random effect	Variance	Std.Dev.	%Variance		
	Cavity ID	1.72	1.31	36.69		

*The significant factor. mR^2 : marginal R^2 , cR^2 : conditional R^2



Figure 4.1: The influence of ALAN (white and green lights) on fledge date and colony departure date, a) shows the correlation between first egg date and fledge date; b) shows the correlation between first egg date and colony departure date. Red= white light, Black: green light and purple= control



Figure 4.2: Weights of young in the treatment groups (white and green lights) and control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates the mean. Whiskers extend to maximum and minimum values; outliers are indicated by filled points.

Chapter 5: As birds age the timing of migration advances and stopover duration decreases in a long-distance migratory songbird

Saeedeh Bani Assadi^{1*}, Kevin C. Fraser¹

¹Department of Biological Science, University of Manitoba

Abstract

The timing of migration can have fitness consequences for long-distance migratory birds, where earlier arrival can bring advantages such as better territories, mates, and access to food resources. In many migratory birds, age plays an important role in migration timing and studies have investigated comparisons of fall and spring migration timing between adults and juveniles. However, how timing may change as birds age has been difficult to study and has received little research attention to date. Here, we tracked the migration timing of individual songbirds (Purple Martins Progne subis), from the ages of 1 to 5, to determine how age influenced migration timing and stopover behaviour in fall and spring migration as well as nest timing and its repeatability. We found that younger birds (two years old) had longer stopover duration during fall migration as well as longer stopovers after crossing a major migration barrier (the Gulf of Mexico) in spring than older birds (greater than two years old), which may be related to reduced foraging and migration efficiency in younger birds. We found that as birds aged, their spring departure and arrival dates, nest timing, and fall arrival dates were earlier, which may relate to shifts in optimal timing as birds age. We did not find that nest timing became more repeatable as birds aged. Overall, our results suggest that experience may influence migratory stopover behaviour and that time selection pressure, particularly in spring, may advance the timing of birds as they age. Our results also indicate that older birds may be better matched with earlier, climate-change driven springs.

Key words: migration phenology, songbird migration, light-level geolocators, aging, repeatability

Introduction

In migratory birds the optimal arrival time, based on game-theoretical models defined by Kokko (1999), is for individuals to arrive at their breeding areas before their competitors and earlier than the optimal time for breeding and foraging. This includes the trade-off where individuals pay the costs of early arrival for the benefits of priority access to territories or mates. Therefore, the timing of spring migration may have important carry over impacts on the fitness of migratory songbirds (Lozano et al. 1996, Kokko 1999). Further, migratory timing or performance could be restricted by conditions experienced before the start of migration (Marra et al. 1998), en route (Tottrup et al. 2012), or by factors such as age (McKinnon et al. 2014, Neufeld et al. 2021). However, there is not enough information about how migratory performance may change as an organism ages (Sergio et al. 2014), particularly for smaller-bodied migrants, where technological limitations have prevented multi-year tracking of individuals across their full migrations.

Improving understanding of age-related patterns may also provide additional insight into how birds may respond to climate change as they age. Most previous studies have focused on differences in migration traits between juveniles and adults (Berthold 2001, Newton 2010, Rappole 2013, McKinnon et al. 2014, Neufeld et al. 2021). There have been very few studies that considered migration traits through years and over full migration routes (e.g., Sergio et al. 2014, Fayet 2020).

It has been demonstrated that young birds often have later arrival time at their breeding grounds as compared to older birds (Morton and Derrickson 1990, Woodrey and Chandler 1997, Hockey et al. 1998, Mueller et al. 2000, McKinnon et al. 2014, Crysler et al. 2016, Neufeld et al. 2021), which may be attributable to endogenous routines that vary by age (Morton and Derrickson 1990, Kokko et al. 2006), morphological differences between age classes (McKinnon et al. 2014), differences in experience (Crysler et al. 2016), or some combination of these factors. For example, juveniles may avoid the costs of early arrival and competition with older birds, where endogenous routines may drive a switch in timing strategy as birds age (Morton and Derrickson 1990, Kokko et al. 2006, Pedersen et al. 2018). For age-related morphological differences, shorter wing size in juveniles can reduce their flight efficiency, making their migrations take longer than for older birds. (McKinnon et al. 2014). Age-related experience may also impact migratory route selection (Crysler et al. 2016), where older birds may have better efficiency in using resources at stopovers, resulting in earlier timing (Hockey et al. 1998), particularly at stopovers close to an ecological barrier (Woodrey 2000). Juveniles may also depart later on their migrations if they spend more time at the breeding grounds than older birds to explore future breeding habitat (Brown and Taylor et al. 2015). Despite these differences between older and younger birds, it is not clear how these changes in migration behaviour have developed or whether they are sudden or gradual. It is also unknown whether age-based changes are based on endogenous cues that change as birds age, or the higher survivability of birds with better performance, or both (Sergio et al. 2014). Most studies to date have relied on comparisons of first-year and after-second year birds and have generally been restricted to patterns at single locations or for segments of migration. The recent development in remote tracking of small animals provides new opportunities to study timing across full migrations as birds age (Alerstam et al. 2006, Stutchbury et al. 2009; Taylor et al. 2017; McKinnon and Love 2018).

We used geolocators to track a long-distance migratory songbird, Purple Martin Progne subis, which breeds across North America and spends the non-breeding season in South America, mainly in the Amazon Basin (Brown et al. 2021, Brown et al. 2021, Fraser et al. 2013). The main goal of this study was to investigate the migration strategies for both spring and fall migrations of a long-distance migratory songbird as they age (1-5 year olds). Our first objective was to investigate potential differences between second-year (SY) and after second-year (ASY) birds in their migration performance such as stopover duration, number of stopovers, stopover duration before and after crossing a major open-water migration barrier, and migratory distance. If SY birds are less efficient at refueling and in route selection owing to lower experience, it was expected that they would have longer migration routes and stopover duration, or more stopovers (Crysler et al. 2016, Yong et al. 1998, Rguibi-Idrissi et al. 2003) as well as a longer stopover duration to refuel after crossing the Gulf of Mexico (Woodrey 2000). The stopover duration and number of stopovers were compared between fall and spring migrations for all SY and ASY birds as it has been shown that the spring migration of birds may generally be faster than fall migration (Nilsson et al. 2013). Our second objective was to investigate whether migration timing changes as birds age (1-5 year old), with the prediction that birds have earlier timing as they age. As birds age they may gain in experience leading to efficiencies that advance timing (Smith et al. 2005), and or selection may favour advanced routines for older birds for both migration and nesting (Hamilton 1966). Therefore, our last objective was to investigate whether nest timing advanced or becomes more repeatable as birds age. We examined the repeatability of individual egg laying date and we predicted individual female purple martins exhibit more repeatability in egg-laying date as they invest more (Langin et al. 2006).

Methods

We used data derived from light-level geolocators that provided daily latitudinal and longitudinal coordinates for each bird throughout the year. Light-level geolocators are small, archival devices that can record the geographical location of a migratory organism by using the timing and intensity of ambient light. Latitude can be determined by day or night length and longitude from the exact time of local midday/midnight (Lisovski et al. 2012). Purple Martin are aerial insectivores which are dependent on human-made houses for their breeding cavities. They have high fidelity to their breeding locations (Brown et al. 2021) that facilitates the retrieval of tracking devices. After trapping using drop-door traps at their nest boxes, geolocators were deployed between 2009-2016 on purple martins at 11 breeding colonies that spanned between 26.1° N and 53.0° N, (supplementary table 5.1). Teflon leg-loop harnesses (Rappole and Tipton 1991; Stutchbury et al. 2009) were used to mount the geolocators. The weight of geolocators (≤ 1.6 g; MK10s/12/12 s/14 s/20, British Antarctic Survey) were not more than 3% of the mean body mass of breeding birds which is ~ 48-55 g (Brown et al. 2021).

A total of 67 tags with annual migration tracks were retrieved in the following year with useable data. These included 33 individuals of different ages that were repeat tracked for 2 years providing the opportunity to compare changes in migratory behaviour as birds age while controlling for individual variation (supplementary table 5.1). The 33 repeated tracked purple martins included 19 females and 14 males. The age of all birds could be categorized as second-year (SY; n=13) and after-second year (ASY; n=54) and sexed based on plumage characteristics (Pyle 1997). A subset of these individuals (n=25) were banded as nestlings and therefore could be aged reliably after their second year (age 2-5 year olds). This subset included 9 individuals that were 3-years old, 4 individuals that were 4-years old, and 2 individuals that were 5 years old.

To analyze the data collected from geolocators, first BASTrak software was used to retrieve the data from the geolocators (Fraser et al. 2012). The *preprocessLight* function from the R-package BAStag (version 0.1.3, Wotherspoon et al. 2016) was used to determine the timing of sunrise and sunset which were defined according to a light-level threshold of 32 and editing or omitting false twilights. Heavy fluctuation in light levels (i.e., a sunrise/sunset event) during the daytime hours at the end of spring migration (in spring of next year of deployment) indicated a bird entering and exiting their nest boxes which was used to determine the arrival date of the bird to the breeding ground (Fraser et al. 2019). Then, the *BAStag2TAGS* function was used to convert the detected twilights in BAStag to TAGS

format to be able to use them in FlightR (0.5.0, Rakhimberdiev et al. 2017). The daily detected coordinates of the bird received after processing the twilight data in FlightR was obtained using the codes provided in the supplementary information (No. 5.2). The timing of departure and arrival in fall and departure date in spring as well as the time of crossing at 23.4° (Tropic Cancer) in both fall and spring migration were determined using changes in the daily detected coordinates of each bird. We used the date that breeding latitude began to consistently decrease to indicate the start of fall migration. The date that latitudes reached below 1° N and where both latitude and longitude remained consistent (within 2°) indicated the wintering arrival date. The spring departure date was determined based on the date that longitudes began to decrease as birds initiated their spring migration by flying westward (Fraser et al. 2013). The date of passing the Tropic of Cancer (23.4°N), which indicated that a bird was approximately at the half-way point on its migration, was determined according to the date that bird was detected at, or closest to, this latitude (Neufeld et al. 2021). Of the 33 repeat-tracked birds, two (from ON) had poor light data for portions of the second year of tracking so we could only include migration data from their first year.

To estimate fall and spring migration distance, the package of "sp" (version 1.4–4, Bivand et al. 2013) was used to obtain arrival and departure dates. The *stationary.migration.summary* function with *min.stay*= 1, from the FlightR package, as well as investigation the detected daily coordinates (where the latitude and longitude were not changed more than 2° for two days or more) (Van Loon 2017) were used to determine stopover timing and duration as well as the number of flight days during fall and spring migrations. We separated analyses for spring and fall because songbirds are known to have different migration strategies in spring and fall (Nilsson et al. 2013).

To investigate whether a bird had stopovers before and after gulf crossing during both fall and spring migrations, we investigated the stopover days of the bird around 27°-30° N (north of Gulf of Mexico) and 18°-20° latitudes (south of Gulf of Mexico). To investigate and control for the impact of distance on the stopover durations before and after barrier crossing, we also measured the flight distance between the stopovers and breeding ground as well as wintering ground.

Nesting data (1995-2014) were acquired through Project Martin-Watch; a citizen science program where nest boxes are monitored 2 or 3 times a week during the breeding season (Purple Martin Conservation Association 2017). The data included first egg date, the age and sex of the birds, and location coordinates. In this study, the nesting data of 84 individuals with nesting data for at least 2 years were used. For 208 of these birds, actual age (1-10 years

old) was known for birds that had been banded as nestlings. These known-age birds included 63 one year olds, 74 two year olds, 36 three year olds, 16 four year olds, 8 five year olds, 3 six year olds, 3 seven year olds, 2 eight year olds, 1 nine year olds, and 1 ten year old (Supplemental table 5.12).

All data collection procedures and experiments were conducted in accordance with the guidelines of the University of Manitoba's Animal Care Committee who have approved this project (Animal Care Protocol Number F18-031/1(AC11388)).

Data analysis:

Influence of age on stopover behaviour

To investigate whether age was a significant predictor of stopover duration (duration of stay, in days) and the number of stopovers during fall and spring migrations, we used linear mixed-effects models (LMMs) fit by REML using the 'lme4' package (version 1.1–19, Bates et al. 2014). In all models, we assigned variables of age class (ASY:SY), sex (male:female), flight distance in fall or spring migration (km), and breeding latitude, as well as an interaction between age and sex as fixed effects. As breeding latitude is an influential factor on migration timing of purple martin in both fall and spring (Neufeld et al. 2021), it was included in all models. The sex and age class, as well as their interaction, were included as males and older birds were predicted to arrive first at the breeding grounds as compared to younger and female birds (Morton and Derrickson 1990). Considering that tagged birds originated from different breeding colonies across the North America flight distances for fall and spring migrations were included in models to control for their likely influence on stopover behaviour. To control for environmental variation in different years of tag deployment, the variable of year was included as a random factor. To control for individual variation and the non-independence of repeated tracks (n=33), individual band number was included as one of the random factors. The locations of breeding colonies were also included as a random effect to control for other potential variation among different breeding colonies. The models were checked for normality in the distribution of residuals and equal variance in the residuals (Zuur et al. 2010). We investigated the influence of outliers in our analyses using Cook's distance and omitted data points from further analyses that were not biologically relevant. We used LM instead of LMM when the random effects did not specify any residual variance. We also compared the stopover duration and number of stopovers between fall and spring migration using t-tests.

Regarding the potential for reduced efficiency of younger birds in flight and refueling at stopovers (Hockey et al. 1998, McKinnon et al. 2014), we predicted that young birds would

have longer stopover duration before and after crossing over the Gulf of Mexico. As the response variables were count data (i.e., stopover days before or after gulf crossing) and included zeros, we fit negative binomial generalized linear mixed-effects models (GLMM) using the 'MASS' package (Venables and Ripley 2002). We first used the 'pscl' package (Zeileis et al. 2008) to investigate whether the zero-inflated negative binomial model was a significant improvement over a standard negative binomial model using the *Vuong* function, but it was not significant. We used GLM instead of GLMM when the random effects did not specify any residual variance. In addition to the fixed effects of age (SY: ASY), sex (male:female), and breeding latitude, we also included the distance between the stopover (before or after the barrier) and the breeding colony and wintering ground to control for the impact of migration distance before or after the stopover on its duration. The random effects of individual, year, and breeding location were also included in the analysis.

We also examined whether age influences the ratio of flying days to stopover days in fall and spring migrations. Owing to the reduced efficiency and experience expected for younger birds, we predicted their ratio of flight days to stopover days would be less than for more experienced, older birds. We fitted LMMs for all of the same variables mentioned above. The normal distribution of residuals and variance equality were checked for our models. We used log transformation for the variable of ratio of flying days to stopover days in fall. We used LM instead of LMM when the random effects did not specify any residual variance.

To test for differences in migratory route between young and old migratory birds (Handel et al. 2010, Crysler et al. 2016), and taking into account the non-normal distribution of these data, we used a Mann-Whitney U test to compare the migratory distance of SY and ASY birds from each breeding colony. Data from birds tracked from sites in New Jersey, Minnesota, and Ontario were omitted from this analysis as there were not any SY birds tracked from these locations for comparison.

Influence of age on migration timing

To investigate changes in migration timing and performance of birds as they age, we fit LMMs with response variables of departure and arrival dates from breeding and wintering grounds as well as dates of crossing the Tropic of Cancer (23.4° N) in fall and spring migrations. For these analyses we again used the same fixed (sex, latitude, and flight distance), and random effects (individuals, breeding locations and year), except here we used the actual age of birds (ages of 23 of purple martins with repeat tracks ranged from 1-5 years old) and not the age categories of SY or ASY. The assumption of normal distribution of residuals and equal variance were checked (Zuur et al. 2010).

Influence of age on the timing of nesting

For the influence of age on the timing of nesting we had two predictions. The first was that older birds (>2 years) would generally have earlier first egg dates, because older birds are expected to have earlier timing generally based upon factors related to age and experience (Morton and Derickson 1990). We therefore expected that birds in their second year (SY) would have later first egg dates than birds that were at least two years old (ASY). Our second prediction was that first egg date would be more repeatable for older individuals (>2 years old), as we expected larger variation between years for younger individuals than more experienced adults. To examine whether age influenced first egg date, we used LMMs with the actual age of 84 individuals from different breeding colonies, sex (male:female), and latitudes as fixed effects and random effects include individuals, year, and breeding colonies. However, the random factor of individual did not show any variance in the residuals. The models for the assumptions of normality of residual distribution and equal variance were checked (Zuur et al. 2010). The repeatability of first egg date for individuals (Shave et al. 2019) was calculated using the package "rptR" (Stoffel et al. 2017) for Gaussian data and was examined separately for each sex. In this analysis, age and latitude were considered as fixed effects and breeding colony, year, and individual ID were set as random effects. Repeatability is expressed between 0 and 1 and a value close to 0 shows high variance within individuals, or low repeatability, and vice versa (Nakagawa and Schielzeth 2010). Parametric bootstrapping with 1000 replications was used to estimate confidence intervals for repeatability.

In all analyses the collinearity among the predictor variables were measured using VIF, which was less than 2 for all variables. We used Akaike Information Criteria corrected for small sample size (AICc) (Burnham and Anderson 2002) through the package "MuMIn" (Barton 2019). We selected the best model among the competitive models with Δ AICc < 2 (supplementary tables 5.2-5.11) using the highest value of the marginal R² (the fixed effects variance) and the conditional R² (the whole model variance) (Barton 2019).

All random effects of each model were investigated first to make sure the models converged with random effects. Therefore, we used likelihood ratio tests to determine whether the significance of the random effects met the model parsimony. Therefore, in each analysis where random effects were insignificant, we did not use the mixed model analysis.

Results:

The influence of age on stopover duration and the number of stopovers:

The longest stopover duration during fall migration was 70 days (ASY male from breeding colony in Alberta) and the shortest stopover was 10 days (ASY male from breeding colony in Virginia). In spring, the longest stopover duration was 25 days for an ASY female from the breeding colony in Virginia, and the shortest stopover duration was 5 days (ASY male with the longest stopover duration in fall). Overall, the greatest number of stopovers (i.e., the number of stops, not their duration) in fall and spring were 13 and 11, respectively, which were made by two ASY females from the Alberta breeding colony.

The mean of stopover duration in fall (47.52 \pm 1.9) was more than in spring (22.86 \pm 0.7) (t = 11.84, df = 83.05, p = 0.00). Also, purple martins had more stopovers during fall (6.24 \pm 0.29) than spring migration (4.6 \pm 0.21) (t = 4.52, df = 116.29, p = 0.00) (Supplemental Fig. 5.1a & b). Totally, the mean number of stopovers and their duration were greater in fall than spring for both age categories (SY and ASY) (Supplemental Fig. 5.1a & b). The lowest number of stopovers in fall was 2 (ASY male from Virginia breeding colony). In spring migration, the lowest number of stopovers was 2 (6 birds). One of these birds was a female from Virginia that had just 2 stops during her spring migration for two years in a row (total 9 days in duration as an SY and total 8 days duration as an ASY).

During fall migration, SY martins had longer stopover duration $(6.21 \pm 2.73 \text{ days}, 95\%$ CI 8.58 to 11.56) than ASY birds (Fig. 5.1). As expected, flight distance between breeding and wintering grounds had positive impacts on total stopover duration and the number of stopovers during both spring and fall migrations where longer distances required more stopovers days and more stops (Table 5.1). However, there were no significant differences in stopover duration between SY and ASY birds in spring (Fig. 5.2).

There was not significant difference in migratory distances in fall and spring migrations between SY and ASY birds for each colony (Fig. 5.3a &b, supplementary table 5.11).

The influence of age on total stopover duration before and after crossing a migration barrier:

Birds had longer stopover duration in fall before and after crossing the Gulf of Mexico as compared to spring. The longest stopover duration before crossing over the Gulf of Mexico in fall was 23 days for a SY female from a Virginia breeding colony. In spring migration, the longest stopover duration before crossing over the Gulf of Mexico was 8 days for a SY female from a. Manitoba breeding colony. The longest stopover duration after crossing over the Gulf of Mexico in fall was 15 days that belong to an ASY female from a Manitoba breeding colony, while the most stopover days after gulf crossing in spring was 5 days for a SY female from a Virginia breeding colony.

Younger, SY birds had longer total stopover duration after gulf crossing in spring (0.88 \pm 0.43, 95% CI 0.03 to 1.73) as compared to older (ASY) birds. Total stopover duration after gulf crossing in spring was also longer for males (0.75 \pm 0.35 (95% CI 0.06 to 1.44) as compared to females (Table 5.2).

The influence of age on the ratio of flying days to stopover days during spring and fall migration:

Differences in flight ratio between SY and ASY birds were not significant for both fall and spring migrations (Table 3). The highest ratio of flight days to stopover days in fall was for an ASY female from a Manitoba breeding colony (26:21). The lowest flight ratio in fall (3:40) and the most flight ratio in spring (16:5) was for an ASY male from Alberta breeding colony. The lowest flight ratio in spring was 3:19 for a SY female from Virginia.

The influence of age on migration timing:

Our results show that spring departure and arrival dates were advanced as birds aged (Tables 5.5, Fig. 5.5, and supplemental Fig. 5.2). Birds at age 2, 3, 4 and 5 began their spring migration 6.77, 14.73, 14.14 and 18.75 days earlier, respectively, than 1 year old birds. Moreover, 2, 3 and 4 year old birds arrived at the breeding ground 8.37, 7.83, and 10.18 days, respectively, sooner than 1 year old birds (Table 5.5, Fig. 5.5, and supplemental Fig. 5.2). Fall arrival date also advanced as birds aged. Two and 3 year old birds arrived at the wintering ground 7.93 and 12.55 days, respectively, sooner than 1 year old birds (Table 5.4, Fig. 5.4, and supplemental Fig. 5.2). As expected breeding latitude impacted timing, where birds breeding further north were later on fall and spring migration (Table 5.4 & 5.5, Fig. 5.4 & 5.5, and supplemental Fig. 5.2).

The influence of age on nest timing and repeatability:

The nest timing of 50 females and 34 males from 16 breeding locations with age range between 1 to 10 years old, revealed an important influence of aging on nest initiation date (Table 5.6; Fig. 5.6). Individuals advanced their nest timing each year as they aged from 1 to 5 years old. However, after 5 years old, the timing of nesting did not continue to advance as birds aged. (Table 5.6). First egg date did not become more repeatable as birds aged; considering other categories (random and fixed effects), the variance was explained by individuals was 0, while most of the variances were explained by fixed factors of age and latitudes and random factor of breeding colony. The repeatability of egg-laying date was higher for females 0.684 (95% CI 0.532 to 0.787), as compared to males (0.295, 95% CI 0.00 to 0.546) (Table 5.7).

Discussion

We examined migration timing and performance of a long-distance migratory songbird as birds aged (1-5 year olds) using direct tracking data across their full migrations. Overall, as birds aged we generally found that the timing of migration and egg laying advanced, and that stopover duration decreased during fall migration and after barrier crossing in spring.

The influence of age on stopover duration and the number of stopovers:

We found that SY birds had longer total stopover duration in fall migration than ASY birds, while this difference was not observed in spring migration (Table 5.1, Fig. 5.1). The fall results align with our predictions and previous studies showing that younger birds spend more time at stopovers. For example, juvenile Ipswich Sparrows (*Passerculus sandwichensis princeps*) had more and longer stopovers than adults, which Crysler et al. (2016) attributed to lower foraging efficiency at stopovers for juveniles, owing to a relative lack of experience. McKinnon et al. (2014) showed that despite using similar spring migration routes, juvenile wood thrushes (*Hylocichla mustelina*) had more stopover days than adults, which could be attributed to their shorter wings resulting in a higher energetic cost of flight, requiring more time at stopovers to refuel.

In addition to age and experience contributing to differences between adults and juveniles, selection may have favoured later timing of juveniles in spring to avoid the costs of earlier arrival and competition with older adults. Such a strategy may result in younger birds spending more time *en route*, maximizing refueling time at stopovers before arrival at the breeding grounds to support reproduction (Alerstam 2006). In the absence of these pressures in fall, higher total stopover duration of SY purple martins in fall could be due to the lower efficiency of SY birds in refueling at a stopover, as stopover duration has positive relationship with refueling rate in migratory songbirds (Yong and Moore 1993; Schaub et al. 2008). Also, as juveniles may arrive at stopovers with less energy, requiring more time to deposit enough energy to continue their migration (Schaub et al. 2008).

As predicted, we found generally that longer migrations required a greater number of stopovers (Table 5.1). Older or younger birds travelling to the same destination did not differ in the length of their routes (Supplemental table 5.11). This does not align with our prediction that younger birds have longer migrations, owing to lower efficiency in their route selection. For example, juvenile Ipswich Sparrows due to lack of enough experience, chose different migration routes than adults, selecting shorter flights over open-water barriers, resulting in longer fall migration overall (Crysler et al. 2016). Longer flight distance requires that small songbirds stopover more frequently, or prolong their stopover duration to refuel to support their continuing migration. As the study of Alerstam (2001) showed, long distance flights

require carrying a greater fuel load which has a higher energetic cost. Therefore, migratory birds may avoid these costs by dividing their flight to several shorter flights to reduce the amount of fuel deposition (Alerstam 2001). The lack of differences in flight distance in fall and spring migration between SY and ASY martins could be due to their dependency on their inherited migration program and the important genetic control of migration routes and distance in passerines (Liedvogel et al. 2011, Berthold 1996; Pulido 2000, Pulido & Berthold 2003, Pulido 2007).

The influence of age on total stopover duration before and after crossing a migration barrier:

As crossing over an ecological barrier poses a lot of risk to migrants (Thorup et al. 2007), it was expected both adults and juveniles would have longer stopovers at the Gulf of Mexico to refuel and/or to wait for favourable conditions to cross (Crysler et al. 2016). We found SY birds had longer stopover duration than ASY birds after crossing over the water barrier in spring migration (Table 5.2), while in fall they did not spend time at stopover after crossing the Gulf of Mexico. The reason for this difference in spring could be due to younger birds delaying arrival at breeding sites to avoid competition with older birds for resources such as food, nests, and mates and increasing survivability encountering harsh weather in early spring (Kokko 1999). This is consistent with the results of a study of Morton and Derrickson (1990) showing that younger purple martins arrived later in spring than older birds, which the authors attributed to selection on timing that reduced the cost of competition, predation, and encountering inclement weather. The lack of any differences in stopover duration before and after crossing the Gulf of Mexico between SY and ASY birds in fall migration, could be due to the lack of competition in arrival dates at the wintering ground in this species, where birds are not territorial and roost in large flocks (cite BNA).

The influence of age on the ratio of flying to stopover days during spring and fall migration:

We expected that if younger birds are less efficient in foraging and have higher energetic costs for migration (Ellegren 1991, Woodrey 2000, Yosef and Wineman 2010)., then they would have a lower ratio of flight to stopover days during migration. However, we did not find differences in flight ratio (flight days to stopover days) between SY and ASY for either fall or spring migration (Table 5.3). In optimal migration models, migratory birds minimize their migration duration and energy costs (Alerstam and Lindstrom 1990). A migratory bird with less fat deposition will generally stay for longer at a stopover (if there are enough food resources) (Alerstam and Lindstrom 1990). Adult migratory passerines tend to use a time-

minimization migration strategy in both spring and fall migration and decrease their stay at stopovers (Yohannes et al. 2009). However, the differences in foraging and accessing to resources between age groups could be negligible if the stopover has enough food resources (Moore and Yong 1991, Seewagen et al., 2013, Schmaljohann et al., 2016). Also, the study of Beauchamp et al. (2020) showed no difference in fat deposition rate between SY and ASY of white-throated sparrow (*Zonotrichia albicollis*) at stopover during spring migration. Despite longer stopover duration of SY birds during fall migration, we found no differences in the ratio of flight days to stopover days between SY and ASY birds. It is possible that SY birds have more flight days due to their smaller wing size and lower flight efficiency (Bowlin 2007, de la Hera et al. 2014), which influences their ratio of flight days to stopover days.

The influence of age on migration timing:

Studies of age-related timing in songbirds have generally shown that SY birds have later timing than ASY birds, including purple martins (Neufeld et al. 2021). However, we investigated timing beyond these broad age categories using banded birds of known age (1 to 5 years old) to determine if birds continue to advance their timing as they get older. Our results showed that individuals continue to advance in the timing of their spring migration and fall departure date as they age, but this pattern generally diminishes as birds approach five years of age. With the exception of spring departure date, where 5-year olds were earliest (Tables 5.4 & 5.5, Fig. 5.4 & 5.5, and supplemental Fig. 5.2).

Fall arrival date at the wintering grounds advanced until age 3. This could be related to longer stopover duration of SY birds in their fall migration. After age 3, we found that birds tended to have later fall and spring arrival dates, although this was not significant. Later timing for older birds could be the result of decreasing of flight efficiency due to reduction in wing length (Møller and De Lope 1999. Piliczewski et al. 2018). A study by Møller and De Lope (1999) showed increasing wing lengths of barn swallow (*Hirundo rustica*) from age 1 to 3, followed by a decrease which they inferred limits flight and foraging efficacy in older birds. This morphological change also has been demonstrated in European blackbird (*Turdus merula*) where wing length decreases after 6 years of age (Piliczewski et al. 2018)

In this study, we did not have data on the very first migrations of young birds, as we began tracking birds at their breeding sites when they were in their second year, after they had already completed their first fall and spring migration. Consequently, the SY birds we measured may not have spent as much time as first-year birds to become familiar with their breeding colony (McKinnon et al. 2014) or accumulating energy for migration (Schmaljohann et al. 2018a). Therefore, the lack of difference in flight distance and fall
departure timing between SY and ASY birds in fall migration, could be related to the experience already gained by second-year birds. However, due to weaker selection on fall migration timing as compared to spring (McKinnon et al. 2016; Alerstam 2006), resulting in fall migration phenology being generally less firm than spring migration phenology (Thorup, et al. 2007; Gallinat et al. 2015), it is generally expected that age related patterns would be weaker in fall than in spring (Mills 2005).

Overall, our study results show an advancement in spring departure and arrival dates by aging and longer stopovers for younger (SY) birds after crossing over a migration barrier, while there were not age-related differences in other migratory behaviours such as spring stopover duration, the number of stopovers, the ratio of flight to stopover days, and migration distance. Our results and those of Morton and Derrickson (1990) where breeding arrival timing advances as purple martins age, could be explained by game theory models, where time selection pressure results in purple martins arriving at the breeding ground at the optimal time for their age (Kokko 1999). Younger birds may avoid the costs of early arrival (energetic and survival costs) at breeding sites when they may likely be outcompeted by older birds (Kokko 1999, Morton and Derrickson 1990).

The influence of age on nest timing and repeatability:

Our results showed that the timing of nesting advanced as birds aged, but then plateaued or became later, potentially due to senescence. We observed that egg-laying date generally advanced until birds were 4 years old, then became more variable (Tables 5.6; Fig. 5.6). Egg laying dates could be driven by the timing of migration, where earlier arrival results in earlier nesting date. However, the study of Morton and Derrickson (1990) showed that early nest timing does not correlate with early arrival date of purple martin. Migration timing and the timing of breeding may also be uncoupled in tree swallow (*Tachycineta bicolor*), where the interval between arrival date of late females and their nesting was shorter than for females that arrived earlier (Stutchbury and Robertson 1987). More variable or later nest dates for martins greater than 5 years old, could be related to their senescence and related morphological changes which influence their performance (Piliczewski et al. 2018). Nesting effort may be higher at intermediate ages due to increasing mortality with senescence later nest timing in the youngest cohort of birds may be connected to the lower efficiency of young birds in accumulating food for reproduction (Harvey et al. 1988).

We expected higher repeatability of egg-laying date in older females (Hochachka 1993, Bańbura and Zieliński 1998), but did not find an age-related role in repeatability. The consistency of egg-laying dates may be mostly governed by the breeding location which could be related to stability of environmental features or phenology at the breeding grounds (Newton 2010, Shave et al. 2020). A study by Thorley and Lord (2015) showed flexibility in the timing of reproduction of Pied Flycatcher (*Ficedula hypoleuca*) in response to advancement in spring, resulted in higher population-level variability, leading to low repeatability of breeding timing. However, without considering the other covariates, females despite their aging, were more repeatable in first egg date which is in consistent with study result of Both et al. (2016) which showed greater consistency in migratory behaviour of females is more than males. Higher repeatability in first egg-dates for females, the ones with actual egg laying ability, could be due to more direct individual control of the timing of egg laying at a finer temporal scale and the greater energy contribution of females than males to reproduction (Langin et al. 2006). Also, females endogenous timing and physiology are influential on egg-laying date (te Marvelde et al. 2012, Salis et al. 2019).

Conclusion:

We investigated migration timing strategies by age in a long-distance migratory bird. We found advancement of spring departure and arrival dates as well as their egg-laying date as birds age which may be tied to age-related differences in innate timing programs. We found that age-related experience did not seem to impact spring migration behaviour but may have influenced stopover duration in fall and after crossing a migration barrier in spring. Our results show that older birds with more advanced spring timing may have the highest fitness with the advancement in spring phenology due to climate change which could be investigated in future research.

Acknowledgements:

Funding and other supports were provided by the University of Manitoba and the NSERC Discovery Grant Program. For geolocator tracking we thank Nanette Mickle, Paul Mammenga, Tim Shaheen, Kelly Applegate, Michael North, Larry Leonard, Edward Cheskey, Megan McIntosh, Pat Kramer, Cassandra Silverio, Lee Bakewell, Richard Doll, Myrna Pearman, James Ray, Alisha Ritchie, Bridget Stutchbury, and John Tautin. We also thank Joe Siegrist from the PMCA and all of the citizen scientists for taking the time to collect the field data for the Project MartinWatch program.

References:

- Alerstam, T. (2001). Detours in bird migration. Journal of Theoretical Biology, 209(3), 319-331.
- Alerstam, T. (2011). Optimal bird migration revisited. Journal of Ornithology, 152(1), 5-23.
- Alerstam, T. (2006). Strategies for the transition to breeding in time-selected migration. *Ardea*, 94(3), 347-357.
- Alerstam, T., Hake, M., & Kjellén, N. (2006). Temporal and spatial patterns of repeated migratory journeys by ospreys. *Animal Behaviour*, 71(3), 555-566.
- Alerstam, T., & Lindström, Å. (1990). Optimal bird migration: the relative importance of time, energy, and safety. In *Bird migration* (pp. 331-351). Springer, Berlin, Heidelberg.
- Bańbura, J., & Zieliński, P. (1998). Timing of breeding, clutch size and double-broodedness in Barn Swallows Hirundo rustica. *Ornis Fennica*, 75, 177-183.
- Barkham, P. (2018). State of the World's Birds. Phys Rev Lett. https:// doi. org/ 10. 1103/ PhysR evLett. 74. 2694
- Bani Assadi, S., Mckinnon, E., Cheskey, E., & Fraser, K. (2021). Does hatch date set the clock? Timing of post-fledging movements for families of a colonially breeding, long-distance migratory songbird. *Journal of Avian Biology*. (under published)
- Barton, K. (2019). MuMIn: multi-model inference, R package version 1.43.15. <u>https://CRAN.R-project.org/package=MuMIn</u>
- Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.
- Beauchamp, A. T., Guglielmo, C. G., & Morbey, Y. E. (2020). Stopover refuelling, movement and departure decisions in the white-throated sparrow: The influence of intrinsic and extrinsic factors during spring migration. *Journal of Animal Ecology*, 89(11), 2553-2566.
- Berthold, P. (2001). Bird migration: a general survey. Oxford University Press on Demand.
- Berthold, P. (1996). Control of bird migration. Chapman and Hall, London, United Kingdom.
- Bivand RS, Pebesma EJ, Gomez-Rubio V (2013) Applied spatial data analysis with R, 2nd edn. Springer
- Both, C., Bijlsma, R. G., & Ouwehand, J. (2016). Repeatability in spring arrival dates in pied flycatchers varies among years and sexes. *Ardea*, 104(1), 3-21.
- Bowlin, M. S. (2007). Sex, wingtip shape, and wing-loading predict arrival date at a stopover site in the Swainson's thrush (Catharus ustulatus). *The Auk*, *124*(4), 1388-1396.
- Brown, J. M., & Taylor, P. D. (2015). Adult and hatch-year blackpoll warblers exhibit radically different regional-scale movements during post-fledging dispersal. Biology Letters, 11(12), 20150593.
- Brown, C. R., D. A. Airola, and S. Tarof (2021). Purple Martin (Progne subis), version 2.0. In Birds of the World (P. G. Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. <u>https://doiorg.uml.idm.oclc.org/10.2173/bow.purmar.02</u>
- Burnham, K. P., and Anderson, D. R. (2002). A practical information-theoretic approach. Model selection and multimodel inference. Springer, New York. doi, 10, b97636.

- Crysler, Z. J., Ronconi, R. A., & Taylor, P. D. (2016). Differential fall migratory routes of adult and juvenile Ipswich Sparrows (Passerculus sandwichensis princeps). *Movement ecology*, 4(1), 1-8.
- de la Hera, I., Pulido, F., & Visser, M. E. (2014). Longitudinal data reveal ontogenetic changes in the wing morphology of a long-distance migratory bird. *Ibis*, 156(1), 209-214.
- Ellegren, H. (1991). Stopover ecology of autumn migrating Bluethroats Luscinia s. svecica in relation to age and sex. *Ornis Scandinavica*, 340-348.
- Fayet, A. L. (2020). Exploration and refinement of migratory routes in long-lived birds. *Journal of Animal Ecology*, 89(1), 16-19.
- Fraser, K. C., Shave, A., de Greef, E., Siegrist, J., & Garroway, C. J. (2019). Individual variability in migration timing can explain long-term, population-level advances in a songbird. Frontiers in Ecology and Evolution, 7, 324.
- Fraser KC, Silverio C, Kramer P et al (2013) A trans-hemispheric migratory songbird does not advance spring schedules or increase migration rate in response to record-setting temperatures at breed- ing sites. PLoS ONE 8:e64587. https:// doi. org/ 10. 1371/ journ al. pone. 00645 87
- Fraser, K. C., Stutchbury, B. J., Silverio, C., Kramer, P. M., Barrow, J., Newstead, D., ... & Tautin, J. (2012). Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. Proceedings of the Royal Society B: Biological Sciences, 279(1749), 4901-4906.
- Gallinat AS, Primack RB, Wagner DL. (2015). Autumn, the neglected season in climate change research. Trends in Ecology & Evolution 30(3):169–176 DOI 10.1016/j.tree.2015.01.004.
- Hamilton, W. D. (1966). The moulding of senescence by natural selection. *Journal of theoretical biology*, *12*(1), 12-45.
- Handel, C. M., & Gill Jr, R. E. (2010). Wayward youth: trans-Beringian movement and differential southward migration by juvenile Sharp-tailed Sandpipers. *Arctic*, 273-288.
- Harvey, P. H., Greenwood, P. J., & Campbell, B. (1984). Timing of laying by the pied flycatcher in relation to age of male and female parent. *Bird Study*, *31*(1), 57-60.
- Hochachka, W. M. (1993). Repeatable reproduction in song sparrows. The Auk, 110(3), 603-613.
- Hockey, P. A., Turpie, J. K., & Velásquez, C. R. (1998). What selective pressures have driven the evolution of deferred northward migration by juvenile waders?. *Journal of Avian Biology*, 325-330.
- Kokko, H. (1999). Competition for early arrival in migratory birds. *Journal of Animal Ecology*, 68(5), 940-950.
- Kokko, H., López-Sepulcre, A., & Morrell, L. J. (2006). From hawks and doves to self-consistent games of territorial behavior. *The American Naturalist*, 167(6), 901-912.
- LACK, D. (1966). Population studies of birds. Oxford Univ. Press, Oxford
- Langin, K. M., Norris, D. R., Kyser, T. K., Marra, P. P., & Ratcliffe, L. M. (2006). Capital versus income breeding in a migratory passerine bird: evidence from stable-carbon isotopes. *Canadian Journal of Zoology*, 84(7), 947-953.
- Liedvogel, M., Akesson, S., & Bensch, S. (2011). The genetics of migration on the move. Trends in Ecology Evolution, 26, 561–569. https://doi.org/10.1016/j.tree.2011.07.009

- Lozano, G. A., Perreault, S., & Lemon, R. E. (1996). Age, arrival date and reproductive success of male American redstarts Setophaga ruticilla. *Journal of avian biology*, 164-170.
- Lisovski S, Hewson CM, Klaassen RHG et al (2012) Geolocation by light: accuracy and precision affected by environmental factors. Methods Ecol Evol 3:603–612. https:// doi. org/ 10. 1111/j. 2041- 210X. 2012. 00185.x
- Marra, P. P., & Holberton, R. L. (1998). Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia*, *116*(1), 284-292.
- McKinnon, E. A., Fraser, K. C., Stanley, C. Q., & Stutchbury, B. J. (2014). Tracking from the tropics reveals behaviour of juvenile songbirds on their first spring migration. *PloS one*, *9*(8), e105605.
- McKinnon EA, Love OP (2018) Ten years tracking the migrations of small landbirds: lessons learned in the golden age of bio-logging. Auk 135:834–856. https:// doi. org/ 10. 1642/ AUK- 17- 202.1
- McKinnon, E. A., Macdonald, C. M., Gilchrist, H. G., & Love, O. P. (2016). Spring and fall migration phenology of an Arctic-breeding passerine. *Journal of Ornithology*, *157*(3), 681-693.
- Mills, A. M. (2005). Protogyny in Autumn Migration: Do Male Birds" Play Chicken"?. *The Auk, 122*(1), 71-81.
- Møller, A. P., & De Lope, F. (1999). Senescence in a short-lived migratory bird: age-dependent morphology, migration, reproduction and parasitism. *Journal of Animal Ecology*, 68(1), 163-171.
- Morton, E. S., & Derrickson, K. C. (1990). The biological significance of age-specific return schedules in breeding Purple Martins. *The Condor*, 92(4), 1040-1050.
- Moore, F. R., & Yong, W. (1991). Evidence of food-based competition among passerine migrants during stopover. *Behavioral Ecology and Sociobiology*, 28(2), 85-90.
- Mueller, H. C., Mueller, N. S., Berger, D. D., Allez, G., Robichaud, W. & Kaspar, J. L. (2000). Age and sex differences in the timing of fall migration of hawks and falcons. Wilson Bull. 112: 214–224.
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, 85(4), 935-956.
- Neufeld, L. R., Muthukumarana, S., Fischer, J. D., Ray, J. D., Siegrist, J., & Fraser, K. C. (2021). Breeding latitude is associated with the timing of nesting and migration around the annual calendar among Purple Martin (Progne subis) populations. Journal of Ornithology, 1-16
- Newton, I. (2010). The migration ecology of birds. Elsevier.
- Nilsson, C., Klaassen, R. H., & Alerstam, T. (2013). Differences in speed and duration of bird migration between spring and autumn. *The American Naturalist*, *181*(6), 837-845.
- Pedersen, L., Jackson, K., Thorup, K., & Tøttrup, A. P. (2018). Full-year tracking suggests endogenous control of migration timing in a long-distance migratory songbird. *Behavioral Ecology and Sociobiology*, 72(8), 1-10.
- Piliczewski, P., Jankowiak, Ł., & Wysocki, D. (2018). Age-dependent changes in biometrics indicate senescence in the European Blackbird Turdus merula. *Bird Study*, 65(2), 219-224.
- Pulido, F. (2000). Evolutionary quantitative genetics of migratory restlessness in the blackcap (Sylvia atricapilla). Marburg: Tectum.

Pulido, F. (2007). The genetics and evolution of avian migration. Bioscience 57, 165-174.

- Pulido,F. & Berthold, P. (2003). Quantitative genetic analysis of migratory behavior. In Avian migration (eds. P. Berthold,E. Gwinner&E. Sonnenschein), pp. 53–77. Springer, Berlin, German
- Purple martin conservation association (PMCA). (2017). Project MartinWatch. https:// www. purpl emart in. org/ resea rch/ 18/ proje ct- marti nwatch/
- Pyle, P. (1997). Identification guide to North American birds: a compendium of information on identifying, ageing, and sexing" near-passerines" and passerines in the hand. Slate Creek Press.
- Rakhimberdiev, E., Saveliev, A., Piersma, T., & Karagicheva, J. (2017). FLightR: an R package for reconstructing animal paths from solar geolocation loggers. *Methods in Ecology and Evolution*, 8(11), 1482-1487.
- Rappole, J. (2013). The avian migrant. Columbia University Press.
- Rappole, J. H., & Tipton, A. R. (1991). New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). Journal of field Ornithology, 335-337.
- Rguibi-Idrissi, H., Julliard, R., & Bairlein, F. (2003). Variation in the stopover duration of Reed Warblers Acrocephalus scirpaceus in Morocco: effects of season, age and site. *Ibis*, 145(4), 650-656.
- Salis, L., Caro, S. P., Hut, R. A., Vernooij, L., & Visser, M. E. (2019). Manipulation of photoperiod perception advances gonadal growth but not laying date in the great tit. *Journal of Avian Biology*, 50(10).
- Schaub, M., Jenni, L., & Bairlein, F. (2008). Fuel stores, fuel accumulation, and the decision to depart from a migration stopover site. *Behavioral Ecology*, *19*(3), 657-666.
- Schmaljohann, H., Meier, C., Arlt, D., Bairlein, F., Van Oosten, H., Morbey, Y. E., ... & Eikenaar, C. (2016). Proximate causes of avian protandry differ between subspecies with contrasting migration challenges. *Behavioral Ecology*, 27(1), 321-331.
- Schmaljohann H (2018b) Proximate mechanisms affecting seasonal differences in migration speed of avian species. Sci Rep 8:4106. https:// doi. org/ 10. 1038/ s41598- 018- 22421-7
- Schmaljohann, H., Mueller, F., Klinner, T., & Eikenaar, C. (2018a). Potential age differences in the migratory behaviour of a nocturnal songbird migrant during autumn and spring. *Journal of Avian Biology*, 49(7), e01815.
- Sergio F, Tanferna A, de Stephanis R, Jiménez LL, Blas J, Tavecchia G, Preatoni D, Hiraldo F (2014) Individual improvements and selective mortality shape lifelong migratory performance. Nature 515:410–413. <u>https://doi.org/10.1038/nature13696</u>
- Seewagen, C. L., Guglielmo, C. G., & Morbey, Y. E. (2013). Stopover refueling rate underlies protandry and seasonal variation in migration timing of songbirds. *Behavioral Ecology*, 24(3), 634-642.
- Schaub, M., Jenni, L., & Bairlein, F. (2008). Fuel stores, fuel accumulation, and the decision to depart from a migration stopover site. *Behavioral Ecology*, 19(3), 657-666.
- Shave, A., Garroway, C. J., Siegrist, J., & Fraser, K. C. (2019). Timing to temperature: egg-laying dates respond to temperature and are under stronger selection at northern latitudes. *Ecosphere*, *10*(12), e02974.

- Smith, R. J., & Moore, F. R. (2005). Arrival timing and seasonal reproductive performance in a long-distance migratory landbird. *Behavioral Ecology and Sociobiology*, 57(3), 231-239.
- Stoffel, M. A., Nakagawa, S., Schielzeth, H., & Goslee, S. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8: 1639– 1644. doi. org/10.1111/2041-210X, 12797.
- Stutchbury, B. J., & Robertson, R. J. (1987). Do nest building and first egg dates reflect settlement patterns of females?. *The Condor*, 89(3), 587-593.
- Stutchbury BJM, Tarof SA, Done T et al (2009) Tracking long-distance songbird migration by using geolocators. Science 323:896. https:// doi. org/ 10. 1126/ scien ce. 11666 64
- Taylor PD, Crewe TL, Mackenzie SA et al (2017) The motus wildlife tracking system: a collaborative research network to enhance the understanding of wildlife movement. Avian Conserv Ecol 12:1–11. https:// doi. org/ 10. 5751/ ACE- 00953- 120108
- Te Marvelde, L., Schaper, S. V., & Visser, M. E. (2012). A single long day triggers follicle growth in captive female great tits (Parus major) in winter but does not affect laying dates in the wild in spring. *PLoS One*, 7(4), e35617.
- Thorley, J. B., & Lord, A. M. (2015). Laying date is a plastic and repeatable trait in a population of Blue Tits Cyanistes caeruleus. *Ardea*, 103(1), 69-78.
- Thorup, K., Bisson, I. A., Bowlin, M. S., Holland, R. A., Wingfield, J. C., Ramenofsky, M., & Wikelski, M. (2007). Evidence for a navigational map stretching across the continental US in a migratory songbird. *Proceedings of the National Academy of Sciences*, 104(46), 18115-18119.
- Tøttrup, A. P., Klaassen, R. H. G., Kristensen, M. W., Strandberg, R., Vardanis, Y., Lindström, Å., ... & Thorup, K. (2012). Drought in Africa caused delayed arrival of European songbirds. *Science*, 338(6112), 1307-1307.
- Van Loon, A., Ray, J. D., Savage, A., Mejeur, J., Moscar, L., Pearson, M., ... & Fraser, K. C. (2017). Migratory stopover timing is predicted by breeding latitude, not habitat quality, in a long-distance migratory songbird. *Journal of Ornithology*, 158(3), 745-752.
- Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0
- Webber, M. I. (1975). Some aspects of the non-breeding population dynamics of the Great Tit (Parus major) (Doctoral dissertation, University of Oxford).
- Woodrey, M. S. (2000). Age-dependent aspects of stopover biology of passerine migrants. *Studies in Avian Biology*, 20, 43-52.
- Woodrey, M. S. & Chandler, C. R. (1997). Age-related timing of migration: geographic and interspecific patterns. Wilson Bull. 107: 52–67.
- Wotherspoon, S., Sumner, M., and Lisovski, S. (2016). BAStag: Basic Data Processing for Light Based Geolocation Archival Tags. Version 0.1.3.
- Woodrey, M. S. (2000). Age-dependent aspects of stopover biology of passerine migrants. *Studies in Avian Biology*, 20, 43-52.

- Yohannes, E., Biebach, H., Nikolaus, G., & Pearson, D. J. (2009). Migration speeds among eleven species of long-distance migrating passerines across Europe, the desert and eastern Africa. *Journal of Avian Biology*, 40(2), 126-134.
- Yong, W., Finch, D. M., Moore, F. R., & Kelly, J. F. (1998). Stopover ecology and habitat use of migratory Wilson's Warblers. *The Auk*, 115(4), 829-842.
- Yong W, Moore FR. 1993. Relation between migratory activity and energetic condition among Thrushes (Turdinae) following passage across the Gulf of Mexico. Condor 95(4):934–943 DOI 10.2307/1369429.
- Yosef, R., & Wineman, A. (2010). Differential stopover of blackcap (Sylvia atricapilla) by sex and age at Eilat, Israel. *Journal of Arid Environments*, 74(3), 360-367.
- Zeileis, A., Kleiber, C., & Jackman, S. (2008). Regression models for count data in R. Journal of statistical software, 27(8), 1-25.
- Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https:// doi. org/ 10. 1111/j. 2041- 210x. 2009. 00001.x

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
	Fixed effects					
	Breeding latitude	0.76 ± 0.41	-4.24	1.57		
Total fall migration	Flight distance in fall migration*	0.002±0.001	9.92	0.004	479.4	0.39/0.46
stopover	Age (SY)*	6.21±2.73	8.58	11.56		
uurauon	Random effect	Variance	Std.Dev.			
	Breeding colony	9.53	3.08			
	Residuals	71.53	8.45			
	Fixed effects					
Total spring migration stopover duration	Flight distance in spring migration*	0.001±0.0003	0.0002	0.001		
	Sex (male)	1.32 ± 1.15	-0.93	3.57	384.20	0.148
uurunom	Age (SY)	2.06 ± 1.41	-0.70	4.83		
	Fixed effects					
Total number	Flight distance in fall migration*	$\begin{array}{c} 0.0005 \pm \\ 0.0002 \end{array}$	0.0001	0.001		
during fall	Breeding latitude	0.09 ± 0.07	-0.05	0.24	265.6	0.34/0.64
migration	Random effect	Variance	Std.Dev.			
	Individuals	1.61	1.27			
	Residuals	1.93	1.39			
Total number	Flight distance in spring migration*	0.0009±0.0001	0.0005	0.001		
of stopovers	Random effect	Variance	Std.Dev.		268.9	0.36/0.62
during spring migration	Individuals	1.45	1.204			
	Residuals	2.09	1.449			

Table 5.1: Top linear mixed models (LMMs) and linear model (LM) for stopover

duration and number of stopovers in fall and spring migration

Table 5.2: Top generalized linear models (GLM) with negative binomial distribution, for

 total stopover duration before and after crossing of the Gulf of Mexico in fall and spring

 migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AIC	R ² (lognormal)
Total stopover duration before gulf crossing in fall migration	Flight distance between the stopover and wintering ground	5.4e-05±7.2e-05	-8.6e-05	0.0001	276.3	0.025
Total stopover duration before gulf crossing in spring migration	Age (SY)	0.31 ± 0.40	-0.46	1.10	220.20	0.106
	Sex (male)	-0.58 ± 0.34	-1.26	0.09	239.39	0.100
Total stopover duration after gulf	Flight distance between breeding ground and the stopover	-0.0002± 0.0002	-0.0006	0.0002		
migration	Breeding latitude	0.08 ± 0.05	-0.009	0.18	266.19	0.097
Total stopover	Age (SY)*	0.88 ± 0.43	0.03	1.73		
duration after gulf	Sex (male)*	0.75±0.35	0.06	1.44	199.05	0.132
migration	Age (SY): Sex (male)	-1.75±0.97	-3.65	0.14		

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	\mathbf{R}^2
Flight ratio in fall migration (log transformed)	Age (SY)	-0.37 ± 21	-0.78	0.04	131.62	0. 04
	Breeding latitude	0.01 ± 0.01	-0.01	0.03		
Flight ratio in spring migration	Age (SY)	$\textbf{-0.24} \pm 0.17$	-0.59	0.11	114.81	0. 08
(log transformed)	Sex (male)	-0.25 ± 0.14	-0.54	0.02		

 Table 5.3: Top linear model (LM) for flight ration (flight days/stopover days) in fall and spring migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR^2/cR^2
	Fixed effects					
Fall	Sex (male)	-1.75±1.95	-5.57	2.06		
departure	Random effect	Variance	Std.Dev.		285.6	0.01/0.80
uate	Individuals	9.81	3.13			
	Breeding location	38.61	6.21			
	Residuals	12.00	3.46			
Fall crossing date of Tropic of Cancer	Fixed effects					
	Breeding latitude*	1.61 ± 0.35	0.91	2.31	315.583	0.37/0.50
	Random effect	Variance	Std.Dev.			
	Individuals	22.63	4.75			
	Residuals	86.20	9.28			
	Fixed effects					
	Breeding latitude*	1.48 ± 0.31	0.87	2.10		
	Age 2*	-7.93 ± 2.53	-12.91	-2.95		
Fall arrival	Age 3*	-12.55 ± 3.38	-19.18	-5.91	27 0 5	0 40 10 74
date	Age 4	-7.81 ± 4.92	-17.46	1.83	350.6	0.49/0.71
	Age 5	-1.27 ± 6.55	-14.13	11.57		
	Random effect	Variance	Std.Dev.			
	Individuals	35.14	5.928			
	Residuals	45.62	6.754			

Table 5.4: Top linear mixed models (LMM) for total fall migration timing

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
	Fixed effects					
	Breeding latitude*	1.32 ± 0.41	0.51	2.12		
	Age 2*	-6.77 ± 1.91	-10.52	-3.02		
Spring	Age 3*	-14.73 ± 2.75	-20.13	-9.32		
departure	Age 4*	-14.14 ± 4.26	-22.50	-5.79		
unit	Age 5*	-18.75 ± 5.69	-29.91	-7.59	336.6	0.51/ 0.86
	Random effect	Variance	Std.Dev.			
	Individuals	53.34	7.30			
	Breeding location	7.76	2.78			
	Residuals	24.79	4.97			
	Fixed effects	·				
Spring	Breeding latitude*	1.46 ± 0.41	0.65	2.27		
crossing date of Tropic of	Sex (male)	-6.13 ± 3.30	-12.61	0.34	295.3	0.46/0.70
Cancer	Random effect	Variance	Std.Dev.			
	Individuals	22.52	4.74			
	Breeding location	12.37	3.51			
	Residuals	44.55	6.67			
	Fixed effects					
	Breeding latitude*	1.88 ± 0.41	1.07	2.69		
	Age 2*	-8.37 ± 2.04	-12.38	-4.35		
Spring arrival	Age 3*	-7.83 ± 2.85	-13.42	-2.23	240 5	0.55/0.06
date	Age 4*	-10.18 ± 4.53	-19.07	-1.29	348.5	0.55/0.86
	Age 5	-7.05 ± 6.06	-18.93	4.82		
	Random effect	Variance	Std.Dev.			
	Individuals	58.80	8.17			
	Breeding location	6.17	2.48			
	Residuals	28.53	5.34			

 Table 5.5: Top linear mixed models (LMM) for total spring migration timing

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
	Fixed effects					
	Breeding latitude	2.52 ± 0.44	1.66	3.39		
	Age 2*	-5.71 ± 1.05	-7.79	-3.64		
	Age 3*	-5.97 ± 1.28	-8.48	-3.45		
	Age 4*	-7.96 ± 1.80	-11.50	-4.42		
	Age 5*	-6.65 ± 2.33	-11.23	-2.07		
Nest timing	Age 6	-3.67 ± 3.56	-10.65	3.30		
	Age 7*	-13.18± 3.49	-20.03	-6.32	1331.6	0.29/0.60
	Age 8	-8.04 ± 4.21	-16.29	0.21		
	Age 9	-6.77 ± 5.86	-18.26	4.71		
	Age 10	-7.52 ± 5.84	-19.16	3.75		
	Random effect	Variance	Std.Dev.	%Variance		
	Breeding location	75.43	8.68	235.82		
	Year	5.27	2.29	16.49		
	Residuals	31.98	5.65			

 Table 5.6: Top mixed models (LMM) for influential factors on nest timing:

Table 5.7: Repeatability scores of first egg date at different levels of year, individual and
breeding colony, where age (actual) and latitude were considered as fixed effects.
Repeatability scores without covariates were also calculated.

•

Factor	Repeatability	95% CI
Repeatability scores of first	egg date considering	covariates
Female		
Individual	0	0, 0.05
Year	0.005	0, 0.041
Breeding colony	0.373	0.153, 0.583
Fixed effects (age +latitude)	0.477	0.276, 0.68
Male		
Individual	0	0, 0.217
Year	0.094	0, 0.307
Breeding colony	0.244	0, 0.519
Fixed effects (age + latitude)	0.239	0.106, 0.506
Repeatability scores of fir	st egg date without co	ovariates
Female		
Individual	0.684	0.532, 0.787
Male		
Individual	0.295	0, 0.546



Figure 5.1. Fall stopover duration comparison between SY and ASY birds



Figure 5.2. Spring stopover duration comparison between SY and ASY birds



3b)

Figure 5.3 A&B. Comparison of flight distance in fall (a) and spring (b) migrations between SY and ASY birds from each breeding colony.





Figure 5.4 A-C. Age-related changes in average departure date (a), crossing date of tropic of cancer (b) and arrival date (c) of fall migration







Figure 5.5 A_C. Age-related changes in average departure date (a), crossing date of tropic of cancer (b) and arrival date (c) of spring migration



Figure 5.6. Comparison of nest timing (1st egg date) of each age group

Chapter 6

Discussion

Taking the 'wild clock' approach in ecological studies provides the opportunity to consider chronotype information alongside studies of environmental input and organismal output (Helm et al. 2017). Using new animal-tracking technology (www.motus.org) in the study of migration timing of purple martin (Progne subis), an aerial insectivore that suffers from population decline particularly in the northern part of its breeding range (Nebel et al. 2010; Smith et al. 2015; Michel et al. 2016), gave me the opportunity to show the important role of nest timing (first egg date) in post-breeding movement timing of juveniles (Chapter 2). My research suggests that nest timing determines the photoperiod experienced by juveniles during nesting development and after fledging and that this is influential on their post-breeding movements. Regarding the "wild clock" approach that was selected in this research, initially, I had included other environmental factors such as temperature and precipitation to investigate their effects on fledge date and colony departure date of adults and juveniles. Regarding the fact that all these stages of post-breeding movements happened within about one month, and all birds experienced the same weather conditions, the first steps of analysis showed the variations of these factors was not significant among different days. Therefore, these covariates were removed from the analysis. I found that later nest timing resulted in a later fledge date and colony departure date. The relationship between nest timing and colony departure date was weaker which could be due to an additional influence of other environmental (in addition to photoperiod) and social factors at this stage. I also found evidence of the 'calendar effect' (Berthold 1988, Berthold 1993) in the colony departure date of juveniles, which could be a response to more rapidly changing daylength experienced after fledging (Chapter 2). However, these results required an experiment to investigate the role of photoperiod in timing further.

To continue my investigations in Chapter 2, I experimentally manipulated photoperiod to further explore the ontogenetic role of photoperiod on post-breeding movement timing of juvenile purple martins in the wild environment (Chapter 3). In response to experiencing a manipulated photoperiod that simulated the day length of an earlier calendar date during nestling development, juveniles showed phenotypic plasticity in the timing of their postbreeding movements through later fledge date and colony departure dates. That the timing of the movement is flexible to photoperiod experienced in the nest suggests its potential use in an assisted evolution approach (Fraser 2021), where an adjustment in the timing of migration based on manipulations in the nest could address the impacts of climate change that can result in a mismatch between food abundance and reproduction phenology (Visser et al. 2004). However, it is still not clear how limited flexible adjustments may be. In other words, determining whether the developed migration timing in the nest would carry over to the future seasons/years will be helpful to better understand how much birds can adjust to new conditions with climate change. Future studies could investigate the efficacy of this method to more rapidly adjust the migration timing of long-distance migratory birds to align with phenological advancement on breeding grounds.

The significant impact of manipulated photoperiod during nesting on their postbreeding movements timing and the impacts of light pollution or anthropogenic light at night (ALAN) (Rich and Longcore 2005; Chepesiuk 2009; Dominoni 2015) encouraged us to consider the impact of ALAN on migration timing of long-distance migratory birds (Chapter 3). As artificial light can be perceived as an extension of photoperiod by birds (Farner, 1964), I conducted an experiment to expose nestlings to different wavelengths of light. I found that exposing nestlings to white ALAN caused later fledge date and later colony departure dates thus revealing the ontogenetic effect of white light on the timing of post-breeding movements of juvenile birds. In contrast, I found that the green ALAN had no impact on their timing.

130

Overall, I demonstrated ontogenetic impacts of photoperiod and ALAN on the post-breeding movements of a long-distance migratory bird in a wild setting as well as the phenotypic plasticity of juveniles to photoperiod and ALAN experienced during their development. As this study showed the disruptive impact of white ALAN with only 5 lux (while the used LEDs in this study did not emit any heat or UV) on migration timing of the experimental birds, and regarding the lack of impact of green ALAN with 5 lux in the wild, using the green or short wavelength of light for illumination of the residential areas close to the breeding colonies of birds could be a potentially effective method for reducing the impact of ALAN. However, further studies are required to examine the carry-over effects of photoperiod as well as ALAN on the circannual rhythm of young migratory songbirds at later stages in their lifecycle.

In addition to my investigation on the impacts of photoperiod as a *zeitgeber* during the nesting period on synchronizing internal clock time with environmental conditions to form migration timing, I examined age as an individual trait which can also impact migration timing, distances, and route (Marra et al. 1993, Newton 2010). Using 33 repeated geolocator tracks of purple martins from eight breeding colonies across North America, I found an advancement in the timing of fall arrival date and spring departure and arrival date as birds aged. I also found a longer total stopover duration for younger (SY) birds on fall migration overall and after crossing a large ecological barrier. Taken together with advancement in fall arrival dates as birds aged, it can be speculated that age and associated experience may play a role in these results. For example, the fall migration behaviour of younger birds may be influenced by reduced flight and foraging efficiency and birds are flexible in fall migration timing. However, advancement in the timing of spring departure and arrival dates, can potentially be explained by game-theory models, where optimal timing through selection favours different timing by age.

131

In addition to differences in migration behaviour by age, I found an advancement in egg-laying date by age that could be the consequence of the advancement in the spring arrival date of older birds (i.e. earlier arriving birds have earlier egg dates). However, I found the egg-laying date could be more flexible to environmental conditions at the breeding colony because of the low repeatability of egg-laying at the individual level. However, females due to more investment in reproduction had higher repeatability in egg-laying dates.

Taken together, my thesis results show the importance of the 'wild clock' approach in ecological studies. I found an important role for nest timing in determining perceived photoperiod by juveniles in a long-distance migratory songbird, which is an important influential *zeitgeber* in timing. Then, for the first time, I experimentally showed the important role of photoperiod, in two forms: a light extension (simulating early date) and ALAN, on the endogenous system of developing nestlings in a wild environment and forming the timing of their post-breeding movements. Further research could consider the carry-over effect of the ontogenetic impact of photoperiod at the juvenile stage on the timing of their future life-cycle events. This thesis also revealed the different fall and spring migration strategies of long-distance migratory songbirds as they age. Overall, my thesis research contributes to the ongoing investigation of the impact of *zeitgebers* within a 'wild clock' approach to better our understanding of factors influencing the timing of a long-distance migratory bird in a changing world.

References:

Berthold, P. (1993). Bird migration: a general survey. Oxford, Oxford University Press

Berthold, P. (1988). The control of migration in European warblers. In XIX Congressus Internationalis Ornithologici (pp. 215-249). University of Ottawa Press.

Chepesiuk, R. (2009). Missing the dark: health effects of light pollution.

- Dominoni, D. M. (2015). The effects of light pollution on biological rhythms of birds: an integrated, mechanistic perspective. *Journal of Ornithology*, *156*(1), 409-418.
- Farner, D. S. (1964). The photoperiodic control of reproductive cycles in birds. *American Scientist*, 52(1), 137-156.
- Fraser, K. C. (2022). Migration in the Anthropocene. In *Songbird Behavior and Conservation in the Anthropocene* (pp. 53-77). CRC Press.
- Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1734), 20160246.
- Marra, P. P., Sherry, T. W., & Holmes, R. T. (1993). Territorial exclusion by a long-distance migrant warbler in Jamaica: a removal experiment with American Redstarts (Setophaga ruticilla). *The Auk*, *110*(3), 565-572.
- Michel, N. L., Smith, A. C., Clark, R. G., Morrissey, C. A., & Hobson, K. A. (2016). Differences in spatial synchrony and interspecific concordance inform guild-level population trends for aerial insectivorous birds. *Ecography*, 39(8), 774-786.
- Nebel, S., Mills, A., McCracken, J., & Taylor, P. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology*, 5(2).
- Newton, I. (2010). The migration ecology of birds. Elsevier
- Rich, C., and Longcore, T. (2005). Ecological Consequences of Artificial Night Lighting. Washington, DC: Island Press.
- Smith, A. C., Hudson, M. A. R., Downes, C. M., & Francis, C. M. (2015). Change points in the population trends of aerial-insectivorous birds in North America: synchronized in time across species and regions. *PloS one*, 10(7), e0130768.
- Visser, M. E., Both, C., & Lambrechts, M. M. (2004). Global climate change leads to mistimed avian reproduction. Advances in ecological research, 35, 89-110.

Appendix:

Supplementary information of chapter 2:

Supplemental Table 2.1: LMM analysis to explain variation in timing of fledge date of juvenile purple martins. The fixed effects were first egg date, number of nestmates, age of parents, weight of nestlings and fat score. The cavity ID was the random effect

Model	Parameter	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	W
	First egg date	1.03	0.08	0.87	1.20			
	Parent age (SY)	1.79	0.81	0.19	3.38			
	Number of nestmates	1.13	0.36	0.42	1.83	244	0.00	0.341
		Variance	SD	%Variance				
Eledge date	Cavity (random factor)	1.37	1.17	44.95				
(Juveniles)	First egg date	1.05	0.08	0.89	1.22			
	Parent age (SY)	1.86	0.80	0.42	1.81			
	Number of nestmates	1.11	0.35	0.28	3.43	245	1	0 156
	Fat score	0.18	0.18	-0.18	0.55			0.150
		Variance	SD	%Variance				
	Cavity (random factor)	1.32	1.15	44.22				

Supplemental Table 2.2: LMM analysis to explain variation in timing of "colony departure date" of juvenile purple martins. The fixed effects were "first egg date", "number of nestmates", and "age of parents". The "cavity ID" was the random effect.

Model	Parameter	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	W
	First egg date	0.52	0.26	0.00	1.04			
		Variance	SD	%Variance		255.1	0.00	0.339
	Cavity (random factor)	15.05	3.88	59.04				
	Null			200.34	205.11			
Colony		Variance	SD	%Variance		256.7	1.6	0.189
(Juveniles)	Cavity (random factor)	18.9	4.34	63.85				
	First egg date	0.52	0.26	0.02	0.77			
	Parent age (SY)	2.14	2.31	-0.01	0.03	256.2	1.2	0 1 4 2
		Variance	SD	%Variance		230.3	1.2	0.143
	Cavity (random factor)	14.45	3.80	58.33				

.

Supplemental Table 2.3: LM analysis to explain variation in age of juvenile at colony departure date. The fixed effects were first egg date, number of nestmates, age of parents.

Model	Models	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	W
Age of	First egg date	-0.46	0.21	-0.88	-0.039	269.168	0.00	0.401
juveniles at colony	First egg date	-0.45	0.21	-0.88	-0.02	270 641	1 47	0.154
departure date	Age of parents	1.19	1.70	-2.14	4.53	270.041	1.4/	0.134



Supplemental Figure 2.1: The panel shows detection data from a receiving station for an individual bird. The colours represent various antennae at the station. Signal strength shows fledging hatch-year purple martin (first stage of post-breeding movement), where F shows initiation of fledging at 18:00 GMT. D shows departure from the colony (second stage of post-breeding movement) on July 30th GMT. The blank spaces demonstrate the moments that the bird left the vicinity of the tower. The signal strength before F shows the time that the bird spent in the cavity.



Supplemental figure 2.2: Comparing fledge date among nestlings of each cavity for 19 of 25 cavities where complete data were available. The size shows the number of nestmates that fledged on the same day.



Supplemental figure 2.3: Comparing first egg date, fledge date and colony departure date of juveniles (n=48, where information on both fledge date and colony departure date were available).



A)





Supplemental figure 2.4a &b: Comparing departure date from breeding colony: among family members of each cavity (n=14) (A), adults (n = 21) vs. juvenile (n = 43), boxes extend to upper and lower quartiles, line indicates median and point indicates mean. Whiskers extend to maximum and minimum values (B).

Supplementary information of chapter 3:



Supplemental Figure 3.1: The day length experienced by nestlings in the experimental, extended day length group (blue line) and in the control group that experienced natural day length (red line) during the nesting period from hatch date until fledge date.





Supplemental Figure 3.2: The panel shows detection data from a receiving station for an individual bird. The colours represent various antennae at the station. Signal strength shows fledging hatch-year purple martin (first stage of post-fledging movement), where initiation of fledging is at 15:20 GMT on 29 July. Departure from the colony (second stage of post-fledging movement) was on 1 August at 11:56 GMT. The blank spaces demonstrate the moments that the bird left the detection vicinity of the receiver tower. The signal strength before fledge date shows the time that the bird spent in the cavity.



Supplemental Figure 3.3: Timing of breeding and post-breeding movements of the control group (black line) and the extended day length group (purple line). The dates represent the mean timing of events.

Supplementary information of chapter 4:

Supplemental Table 4.1: Competitive models resulted from LMM analysis to explain variation in the timing of "fledge date" of juvenile purple martins. The fixed effects were "first egg date", treatments (white and green lights), "number of nestmates", and "weight".

Model	Parameter	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	mR ² /cR ²
	Fixed effects							
Fledge	First egg date*	1.00	0.03	0.92	2.07			
date	Treatment (green light)	0.56	0.36	-0.14	1.27			
	Treatment (white light) *	1.53	0.37	0.78	2.27			
	Weight	0.01	0.02	-02	0.06	545.8	1.4	0.90/ 0.92
	Number of nestmates	0.32	0.15	0.03	0.62			
	Random effect	Variance	SD	%Variance				
	Cavity (random factor)	0.50	0.70	25.32				

The "cavity ID" was the random effect. the random effect.

*The significant factor. mR^2 : marginal R^2 , cR^2 : conditional R^2
Supplemental Table 4.2: Competitive models resulted from LMM analysis to explain variation in the timing of "duration in the nest" of juvenile purple martins. The fixed effects were "first egg date", treatments (white and green lights), and "number of nestmates". The "cavity ID" was the random effect.

Model	Parameter	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	mR ² /cR ²
	Fixed effect							
Duration	First egg date*	0.02	0.03	-0.04	0.09			
in the	Treatment (green light) *	0.73	0.32	0.09	1.37			
nest	Treatment (white light) *	1.63	0.33	0.97	2.30			
(days)	Weight	0.04	0.02	0.002	0.087	531.0	1.5	0.28/0.42
	Number of nestmates	0.31	0.13	0.04	0.58			
	Random effect	Variance	SD	%Variance				
	Cavity (random factor)	0.34	0.58	19.85				

*The significant factor. mR^2 : marginal R^2 , cR^2 : conditional R^2

Supplemental Table 4.3: Competitive models resulted from LMM analysis to explain variation in the timing of "duration in the colony" of juvenile purple martins. The fixed effects were "first egg date", treatments (white and green lights), and "number of nestmates". The "cavity ID" was the random effect.

Model	Parameter	Estimate	Std. Error	95% CI (lower)	95% CI (upper)	AICc	ΔAICc	mR ² /cR ²
Duration	Fixed effect							
in the	First egg date*	0.02	0.03	-0.04	0.09			
nest	Treatment (green light) *	0.73	0.32	0.09	1.37			0.28/0.42
(days)	Treatment (white light) *	1.63	0.33	0.97	2.30			
	Weight	0.04	0.02	0.002	0.087	531.0	1.5	
	Number of nestmates	0.31	0.13	0.04	0.58			
	Random effect	Variance	SD	%Variance				
	Cavity (random factor)	0.34	0.58	19.85				

*The significant factor. mR^2 : marginal R^2 , cR^2 : conditional R^2

Supplemental Table 4.4: ANCOVA analysis of the regression lines between first egg date and fledge date, and between first egg date and colony departure date with considering the treatments of white and green light.

Model		DF	F value	Р
First egg date and fledge date	Treatments	2	85.60	0.00
	First egg date	1	1167.16	0.00
	Treatment: first egg date	2	0.22	0.8
	· _			
First egg date and Colony	Treatments	2	13.94	0.00
departure date	First egg date	1	101.81	0.00
	Treatment: first egg date	2	2.78	0.06



Supplemental Figure 4.1: Schematic of the ALAN treatment. 5 lux of artificial white or green light were turned on at sunset and off at sunrise.



Supplemental Figure 4.2: The panel shows detection data from a Motus receiver station for an individual bird. The colours represent various antennae at the station. Signal strength shows fledging date and time for hatch-year purple martin (first stage of post-fledging movement), with the initiation of fledging on July 24th, at 21:16 GMT and departure from the colony (second stage of post-fledging movement) on July 28th, at 12:00 GMT. The blank spaces demonstrate the moments that the bird left the vicinity of the tower. The signal strength before fledge date shows the time that the bird spent in the cavity.

Supplementary information of chapter 5:

State/Province	Breeding colony	Geolocators retrieved	Latitude	Longitude	Years deployed
Ontario (ON)	Walpole	2	42.61	-82.54	2014-2015
Manitoba (MB)	Private residence	4	49.73	-97.13	2015-2016
	Camrose	2	53.01	-112.86	
Alberta (AB)	Ellis Bird Farm	7	52.39	-113.61	2012-2015
Virginia (VA)	Woodbridge	7	38.61	-77.26	2010-2015
Pennsylvania (PA)	Erie	4	42.12	-80.15	2009-2013
South Dakota	Sioux Falls	1	43.50	-96.70	2011 2014
(SD)	Columbia	4	45.60	-98.31	2011-2014
New Jersey (NJ)	Locust	1	40.39	-74.00	2011-2012
Minnesota (MN)	Brainerd	1	46.40	-94.20	2011-2012

Supplemental table 5.1: Breeding purple martins (*Progne subis*) location and the number of geolocators that retrieved from each location.

5.2.R-code used to extract the detected coordinates of the bird during migration using geolocator data

R < -Result

Lat = R\$Result\$Quantiles\$Meanlat

Lon = R\$Result\$Quantiles\$Meanlon

#Pull Date

Date = as.Date(R\$Results\$Movement.results\$time)

#Pull DOY

DOY = R\$Results\$Movement.results\$yday

#May need to add 1 to the days, if January 1st is 0

for (i in 1:577) { DOY[i] = DOY[i]+1 }

#pull credible intervals

LowerCILat = R\$Results\$Quantiles\$LCI.lat

UpperCILat = R\$Results\$Quantiles\$UCI.lat

LowerCILon = R\$Results\$Quantiles\$LCI.lon

UpperCILon = R\$Results\$Quantiles\$UCI.lon

M=data.frame(Date,DOY,Lat,LowerCILat,UpperCILat,Lon,LowerCILon,UpperCILon)

write.csv(M, file="R.csv")

Supplemental table 5.2: Linear mixed model results for fall migration stopover duration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
	Fixed effects					
	Flight distance in fall migration	3.212e-03 ±9.339e-04	0.001	0.005		
	Age (SY)	6.95±2.73	1.59	12.30	479.9	0.23/0.39
	Random effect	Variance	Std.Dev.	%Variance		
	Breeding colony	19.17	4.379			
	Residuals	71.06	8.430			
	Fixed effects					
	Flight distance in fall migration	0.003 ± 0.0009	0.001	0.005		
	Age (SY)*Sex(M)	-1.18 ± 6.07	-23.73	0.08		
Total fall migration	Sex (male)	1.43 ±2.37	4.07	16.54	480.2	0.28/0.44
stopover	Age (SY)	$10.31{\pm}~3.18$	-10.63	19.96		
duration	Random effect	Variance	Std.Dev.	%Variance		
	Breeding colony	19.81	4.45			
	Residuals	66.67	8.16			
	Fixed effects					
	Latitude	$1.31{\pm}0.29$	0.72	1.89		
	Age (SY)	5.034±2.78	-0.42	10.49		
	Random effect	Variance	Std.Dev.	%Variance	481.2	0.38/0.43
	Breeding colony	6.40	2.53			
	Residuals	77.77	8.81			

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR^2/cR^2
	Fixed effects					
	Flight distance in fall migration	$\begin{array}{c} 0.0009 \\ \pm \ 0.0001 \end{array}$	0.0006	0.001		
	Age (SY)	-0.66 ± 0.55	-1.75	0.41	269.5	0.37/0.61
	Random effect	Variance	Std.Dev.	%Variance		
	Individual	1.31	1.14			
Total number	Residuals	2.11	1.45			
of stopovers	Fixed effects					
migration	Flight distance in fall migration	$\begin{array}{c} 0.0009 \\ \pm \ 0.0001 \end{array}$	0.0005	0.001		
	Age (SY)	$0.24{\pm}~0.50$	-0.75	1/2	270.6	0.36/0.62
	Random effect	Variance	Std.Dev.	%Variance		
	Individual	1.48	1.21			
	Residuals	2.07	1.43			

Supplemental table 5.3: Linear mixed models for stopover numbers in fall migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	\mathbf{R}^2
	Flight distance in spring migration	0.001 ± 0.0003	0.0002	0.001	83.70	0.10
	Flight distance in spring migration	0.001± 0.0003	0.0003	0.001	84 263	0.12
Total	Age (SY)	1.79± 1.39	-0.94	4.53	04.205	0.12
stopover duration in spring	Flight distance in spring migration	0.001± 0.0003	0.0002	0.001	385 11	0.11
migration	Sex (M)	1.04 ± 1.04	-1.20	3.28	565.11	
	Flight distance in spring migration	0.001± 0.0006	0.0002	0.002	205.22	0.11
	Breeding Latitude	-0.001 ± 1.6	-0.45	0.19	385.32	

Supplemental table 5.4: Linear mixed models for stopover duration in spring migration

Supplemental table 5.5: Linear mixed models for stopover numbers in spring migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR^2/cR^2
Total number of stopovers during spring migration	Flight distance in spring migration	0.0005 ± 0.0001	0.0003	0.0007	211.31	0.277

Supplemental table 5.6: Generalized linear models (GLM) with negative binomial distribution for total stopover duration before and after crossing of the Gulf of Mexico in fall and spring migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AIC	R ² (lognormal)
Total stopover duration before gulf crossing in fall migration	The distance between breeding ground and the stopovers	0.0001 ±0.0001	-0.0001	0.0003	276.64	0.022
	Age (SY)	$0.38{\pm}0.39$	-0.38	1.16	243.24	0.024
	Sex (male)	-0.54 ± 0.33	-1.19	0.10	241.66	0.069
Total stopover duration before gulf	Age (SY)	0.29 ± 0.39	-0.47	1.05	243.10	0.08
crossing in spring migration	Sex (M)	-0.50 ± 0.33	-1.16	0.16		
	Sex (M)	-0.58 ± 0.33	-1.23	0.07	243.20	0.086
	Breeding Latitude	0.01 ± 0.02	-0.03	0.07		
Total stopover duration after gulf	Breeding Latitude	0.051 ± 0.03	-0.008	0.11	266.19	0.078
crossing in fall migration	Flight distance between the stopover and wintering ground	8.434e-05± 6.709e-05	-4.7154e- 05	0.0002	267.15	0.045
	Age (SY)*	0.88 ± 0.42	0.06	1.71		
	Sex (male)*	0.67±0.34	0.0001	1.35	105 50	0.11
	Age (SY): Sex (male)	-1.67±0.94	-3.52	0.18	195.50	0.11
	Age (SY)	0.35 ± 0.36	-0.36	1.078	196.50	0.018
Total stopover duration after gulf	Sex (male)	0.28±0.31	-0.32	0.90	196.59	0.018
crossing in spring migration	Flight distance between wintering ground and the stopover	5.231907e-05 ± 6.163535e-05	-6.84	0.0001	196.79	0.016
	Age (SY)	0.52 ± 0.37	-0.20	1.25		
	Sex (male)	0.43±0.31	-0.19	1.05	196.78	0.063

Supplemental table 5.7: Summary of linear model (LM) for flight ration (flight

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	\mathbb{R}^2
Flight ratio in	Flight distance	1.019e-04 ±5.476e-05	-5.427187e-06	0.0002	135.21	0.052
fall migration (log transformed)	Age (SY)	-0.14 ± 0.22	-0.57	0.29	12650	0.057
	Flight distance	9.440511e-05±5.629 685e-05	-1.593468e-05	0.0002	136.79	
	Breeding latitude	0.01 ± 0.01	-0.01	0.04	115.10	0.01
	Age (SY)	-0.19 ± 0.17	-0.54	0.15	114.98	0.01
Flight ratio in spring migration	Sex (M)	-0.22 ± 0.14	-0.50	0.06	113.87	0.03
(log transformed)	Breeding Latitude	0.01 ± 0.01	-0.01	0.03	114.68	0.05
	Sex (M)	-0.22 ± 0.14	-0.50	0.06		
	Age (SY)	-0.25 ± 0.17	-0.60	0.09	110 77	0.06
	Sex (M)	-0.25 ± 0.14	-0.54	0.02	113.77	0.06
	Sex (M)	-0.23 ± 0.14	-0.52	0.053		0.042
	Flight distance	3.146272e-05 ± 4.966853e-05	-0.00006	0.0001	115.45	

days/stopover days) in fall and spring migration

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	$\mathbf{mR}^2/\mathbf{cR}^2$
	Fixed effects					
	Breeding latitude	1.43±0.31	0.81	2.04		
Fall arrival date	Random effect	Variance	Std.Dev.		357.3	0.36/0.49
	Individuals	18.14	4.25			
	Breeding location	1.06	1.03			
	Residuals	77.67	8.81			
4						

Supplemental table 5.8: Summary of linear mixed models (LMM) for total fall migration

timing

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	$\mathbf{mR}^2/\mathbf{cR}^2$
	Fixed effects					
Spring	Breeding latitude*	1.46 ± 0.40	0.67	2.25		
crossing date	Random effect	Variance	Std.Dev.		296.5	0.42/0.69
Cancer	Individuals	31.92	5.65			
	Breeding location	9.09	3.01			
	Residuals	45.39	6.73			

Supplemental table 5.9: Summary of linear mixed models (LMM) for total spring

migration timing

Model		Estimate ± Std. Error	95% CI (lower)	95% CI (upper)	AICc	mR ² /cR ²
Nest timing	Fixed effects					0.28/0.58
	Breeding latitude	1.11 ± 0.33	1.66	3.39	1355.2	
	Sex (M)	0.59 ± 0.90	-1.17	2.36		
	Age 2*	-5.68 ± 1.05	-7.79	-3.61		
	Age 3*	-6.05 ± 1.27	-8.54	-3.56		
	Age 4*	-8.15 ± 1.79	-11.67	-4.64		
	Age 5*	-6.99 ± 2.34	-11.58	-2.39		
	Age 6	-3.66 ± 3.52	-10.57	3.23		
	Age 7*	-13.33± 3.46	-20.12	-6.53		
	Age 8	-8.31 ± 4.17	-16.49	0.12		
	Age 9	-7.08 ± 5.83	-18.52	4.36		
	Age 10	-7.95 ± 5.82	-19.37	3.45		
	Random effect	Variance	Std.Dev.			
	Breeding location	17.25	4.15			
	Year	5.66	2.38			
	Residuals	31.37	5.60			

Supplemental table 5.10: Summary of mixed models (LMM) for influential factors on

nest timing:

Breeding location		Average flight distance (km)	W	p-value
	SY birds in Spring	8721	22	0.47
Alborto	ASY birds in Spring	9349		
Alberta	SY birds in Fall	8204	28	0.11
	ASY birds in Fall	9430		
	SY birds in Spring	8058	0	0.78
Manitaha	ASY birds in Spring	8089	7	
Maintoba	SY birds in Fall	8605	12	0.14
	ASY birds in Fall	9723	15	
	SY birds in Spring	6729	7	0.25
Donnovilvonio	ASY birds in Spring	7009	7	
Pennsylvania	SY birds in Fall	7429	7	0.25
	ASY birds in Fall	7256	/	
	SY birds in Spring	7546	7	0.88
South Dakota	ASY birds in Spring	7387		
South Dakota	SY birds in Fall	8277	Λ	0.4
	ASY birds in Fall	7542	4	
	SY birds in Spring	6543	20	0.59
Vincinio	ASY birds in Spring	6259		
virgima	SY birds in Fall	6444	28	0.76
	ASY birds in Fall	6440		

Supplemental table 5.11: Wilcox test to compare flight distance in between SY and ASY birds from different breeding colonies except breeding colonies of Ontario, New Jersey and Minnesota.

State/Province	Latitude	Longitude	Geolocators retrieved	Years deployed
Florida (FL)	28.35	-81.5876241	1	2013-2014
Manitoba (MB)	49.73476	-97.1303	1	2016-2017
Alberta (AB)	52.39	-113.51	3	2012-2013
Kansas (KS)	37.41	-90.7	1	2017-2018
Pennsylvania (PA)	41.8	-80.0852	32	2007-2019
South Dakota (SD)	45.6	-98.3126	5	2011-2014
New Jersey (NJ)	40.4	-74.0263	9	2011-2012
Virginia (VA)	38.61	-77.15	9	2010-2013
Minnesota (MN)	46.4	-94.1944	24	2011-2020

Supplemental table 5.12. Breeding purple martins (*Progne subis*) location for egg-laying data and the number of geolocators that retrieved from each location.







Supplemental figure 5.1A&B: Comparing the stopover duration and numbers of adults and juveniles of purple martins in fall and spring



Alberta breeding colony





B)







D)



E)

Supplemental figure 5.2 A-E: Comparing different stages of arrival date, departure date and crossing date of Tropic of Cancer in both spring and fall migration timing of purple martins from different colonies of Alberta (A), Manitoba (B), Pennsylvania (C), South Dakota (D), and Virginia (E) at different latitudes.