

**What factors fine-tune timing of egg-laying in  
European starlings (*Sturnus vulgaris*)?**

**by  
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## Abstract

Here I investigated the role of two putative “supplemental cues”, temperature and social factors, on timing of egg-laying in a local population of European starlings, *Sturnus vulgaris*, a highly photoperiodic and semi-colonial nesting species. A long-term temperature signal, spanning January through March, best predicted onset of egg-laying in females though there was significant residual temperature-independent variation. Social factors were only associated with this temperature-independent residual laying date, not absolute laying date. Individual variation in temperature-predicted residual laying date was associated with nearest neighbour distances in a “linear” habitat, network familiarity, and synchrony in laying though was independent of residency and female familiarity. This suggests that temperature provides a relatively long-term cue for timing of egg-laying in European starlings and that some components of social network structure could act as “supplemental cues” to fine-tune timing of laying to the local environment.

**Keywords:** Egg-laying date; Supplemental cues; Female social networks; Temperature; Birds

## **Dedication**

I would like to dedicate this to my very supportive friends and family. For my spouse, Michael Mabary, for all of his support, love, and friendship along this journey. To my family who always believe in me and support me in pursuing my dreams: my mom – Laurie, dad – Bill, brother – Alex, sister – Courtney, grandma – Vicki, and grandpa – Bill. Also to my parents-in-law, Sheila and Quentin Mabary. My best friend, Alex Clarke aka my Khalila. Lastly, a special shout-out to my sister-in-law, Gabrielle Viviano, who has been so strong and brave in her battle against Grade 3 Astrocytoma brain cancer. My greatest wish is for you to beat the odds and stay here with us. I love you all.

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# Preface/Executive Summary

## General introduction

Phenology, or timing of events within an organism's annual cycle, (e.g., migration, pupation, bud burst, invertebrate emergence, pre- and post-nuptial moult, reproduction) is a critical component for an organism's fitness, as experiences at one phase of the annual cycle can affect fitness during subsequent life-history events (Visser et al., 2010). Timing of life-history stages is particularly important for species with annually seasonal environments as resource availability is often limited, and it is important that organisms can accurately predict future conditions that optimise survival of both parents and offspring. This is because the environment for 'decision' making is often spatially and/or temporally separated from the environment where the selection occurs on the output of that decision, such as offspring survival (Farner, 1986; Visser et al., 2010). Organisms that depend on seasonally available resources face risk of "mismatch" if they respond to environmental change at different rates than the resources they depend on (Walker II et al., 2019). Therefore, it is important that organisms are able to accurately time life cycle events to match environmental conditions that optimise fitness at the 'environment of selection' (Visser et al., 2010). In a wide range of taxa, photoperiod provides the "initial predictive information" needed to initiate hormonal processes at roughly the same time each year and therefore is a reliable cue for broad seasonal rhythms of the annual cycle (Walker II et al., 2019). It is then thought that "supplemental cues", such as temperature, vegetation characteristics, prey abundance, and social cues, predictive of local conditions fine-tune timing of life-cycle events further contributing to 'decisions' on timing (Visser et al., 2010).

Global climate change effects on phenology, and potential mismatches at different stages of an organism's annual cycle, has been a topic of interest for many years (Caro et al., 2013), however our knowledge of supplemental cues for timing, other than temperature, is patchy. Temperature-dependent processes provide important resources for organisms at higher trophic levels (i.e., seed germination, leaf production, flowering, insect development), with many of these processes associated with winter climatic conditions, which can cause significant carry-over effects to reproductive timing and success of organisms depending on temperature-dependent resources (Carey, 2009; Saino et al., 2004; Selonen et al., 2021; Visser et al., 2009). For example, in red

deer (*Cervus elaphus*) warmer temperatures were associated with earlier birth dates and body mass, and higher rainfall with reduced juvenile survival and female fecundity (Stopher et al., 2014). Reduced quality or abundance of temperature-dependent resources can have multi-trophic level consequences, for example Saino et al. (2004) found that primary productivity, or vegetation biomass, in the winter (Dec – Feb) affected different life history processes related to reproductive success in barn swallows (*Hirundo rustica*), such as wing and tail length during prenuptial moult. Additionally, low vegetative biomass on winter grounds resulted in delayed laying and lower fledgling success in comparison to years of high quality (Saino et al., 2004). In another study, Pearce-Higgins et al. (2010) found that temperatures in August were associated with crane-fly (Tipulidae; a prey species of Eurasian golden plovers, *Pluvialis apricaria*) abundance the following year, with warmer temperatures leading to reduced population size. This was associated with the productivity of golden plovers two years later which further demonstrates how multi-trophic level interactions can cause carry-over effects on phenology and productivity throughout the annual cycle.

Breeding phenology in birds has been well-studied, as birds have broadly predictable annual cycles, consisting of migration, pre- and post-nuptial moult, and reproduction occurring at roughly the same time each year (Williams, 2012). Additionally, as the final stages of gonadal maturation in females occurs just prior to onset of egg-laying, females are thought to integrate a broad range of environmental information from non-photic supplemental cues to fine-tune timing of egg-laying within the broader reproductive window determined by day length (Hau et al., 2008; Farner and Wingfield, 1980). Despite social interactions being thought of as supplemental cues for over eighty years (Darling, 1938), relatively little progress has been made in understanding how social dynamics relate to reproductive timing (cf. synchrony). Research on social factors for timing of egg-laying in birds has mainly focused on the roles of male displays (courtship, singing) despite the fact that females are the sex that controls the egg-laying decision (Williams, 2012). Importantly, given the strong influence of temperature there appear to have been no studies examining the relationship between social structure and temperature-independent laying (i.e., residual variation). In this thesis, I investigate how temperature and female social networks (neighbour distances, residency, female -and- network familiarity, and synchrony) might act as supplemental cues to time breeding in a local population of European starlings (*Sturnus vulgaris*).

### **Study species**

The European starling is a well-studied, highly social, semi-colonial nesting species that is commonly found in urban and agricultural environments where there is easy access to food resources and high availability of nesting cavities or man-made nest-boxes. Starlings congregate in large flocks in late summer, fall, and winter and are very tolerant of other birds. Starlings remain in small flocks during the spring and breeding season and will nest as close as 0.5m to each other (Feare, 1984; Kessel, 1957). At the start of the breeding season, males select, defend and prepare a nesting site (in addition to displaying via song, posture, and behaviour; Pavlova et al., 2010) to solicit females to mate with them. Once mated, females will lay one egg each day up until clutch completion, laying an average of 5 eggs in peak broods (T.D. Williams, unpub. data). Starlings are highly synchronous in egg-laying and generally more than 80% of females in a population begin laying within 5 days after the first egg of a local population is laid (Tinbergen, 1981) though there can be variation between different populations and within a population (Smith, 2004). The female begins incubation upon clutch completion for ~11 days until hatching of chicks which fledge ~21 days later. Photoperiod regulates hormones associated with seasonal changes in behaviour and physiology (e.g., bill colour, song, gonadal maturation) and annual variation in egg-laying is associated with temperature differences at a given locality (Kessel, 1957). Female starlings sing long, elaborate songs in and out of the breeding season which is suggested to serve the same function of male song (i.e., competition mediation, predator defence, environmental information) (Pavlova et al., 2010). As starlings are highly social and photoperiodic organisms, they make a great study species for examining how supplemental cues are associated with timing of egg-laying.

### **Thesis objectives**

In the first chapter of this thesis, I re-assess the relationship between ambient temperature, a putative supplemental cue, and timing of egg-laying in European starlings using three complimentary approaches: an 'unconstrained' analysis (climwin), a more traditional sliding window analysis, and analysis of specific, biologically-informed temperature windows. In my second chapter, I used the temperature-predicted timing of egg-laying to examine the relationship between social network structure and variation in timing of egg-laying at a population and individual level to determine if, and how, five

female social network metrics (neighbour distances, residency, female -and- network familiarity, and synchrony) might act as supplemental cues to fine-tune timing of egg-laying.

# Chapter 1. Temperature and timing of egg-laying in European starlings (*Sturnus vulgaris*)

## Abstract

Timing of egg-laying by female birds has important consequences for fitness, as seasonal availability of food is often limited. So, it is important females accurately predict, often weeks to months in advance, when resources will be most abundant at the time of chick-rearing. In many avian species, timing of breeding is relatively predictable across years as photoperiod provides “initial predictive information” needed to initiate seasonal reproductive development, thus timing breeding within a broad photoperiodic window. It is thought that females then use non-photic “supplemental cues” (i.e., short-term temperatures, food, social factors) to fine-tune timing of egg-laying to local conditions. Here, we re-assessed the relationship between ambient temperature and timing of egg-laying in European starlings (*Sturnus vulgaris*) using ‘unconstrained’ exploratory, traditional, and confirmatory approaches to examine how, and over what time period, temperature might act as a supplemental cue to fine-tune timing of egg-laying. We found that temperature provides a relatively long-term cue for timing of egg-laying, with temperatures from mid-winter (January/February) to the immediate pre-breeding period (March) being most informative of variation in laying date. This provides little support for the key idea that short-term, pre-laying, temperatures act as supplemental cues to fine-tune egg-laying to local conditions at the time of egg-laying. Rather, birds appear to integrate temperature information over a relatively long-term period leading up to egg-laying.

**Keywords:** Egg-laying date; Supplemental cues; Females; Temperatures

## Introduction

Timing of breeding by female birds, and specifically timing of egg-laying, has important consequences for fitness (Williams, 2012). Birds time their breeding to seasonal peaks of food availability which are often of limited duration. Physiological and behavioural preparation for breeding can take weeks to months and birds must therefore be able to predict onset of breeding which they do using information from environmental

cues or “proximate” factors (Farner and Wingfield, 1980; Visser et al., 2009, 2012; Wingfield et al. 1997). Photoperiod provides the “initial predictive” information needed to initiate migration to the breeding grounds and seasonal reproductive development (Dawson, 2008; Dawson et al., 2001; Sharp, 1996). Both sexes respond to increasing day length with a “switch” on of upstream components of the reproductive axis (the hypothalamus and pituitary) (Hau et al., 2008; Perfito et al., 2015; Williams, 2012). In response to increasing daylength in spring, males show a slow, gradual, increase in testis size and function (spermatogenesis, steroidogenesis) over several weeks or months prior to breeding with daylength being a necessary, and perhaps sufficient, cue for male reproductive development. In contrast, in females the critical final stages of gonadal maturation occur just before onset of egg-laying involving estrogen synthesis and secretion by the ovary, estrogen-dependent onset of vitellogenesis, a shift in lipid metabolism to yolk-targeted very low-density lipoproteins in the liver, rapid yolk development, and oviduct development (Williams, 2012; Wingfield et al., 1997). It is becoming accepted that female gonadal maturation is therefore regulated mostly downstream in the hypothalamic-pituitary-gonadal axis at the level of the ovary and liver (Ball, 2008; Perfito et al., 2015; Verhagen et al., 2019; Williams, 2012). Furthermore, this final step of gonadal maturation in females is thought to involve integration of a broader range of environmental information from non-photoc “supplemental cues” (temperature, food, social factors) to fine-tune timing of egg-laying within the broader reproductive window determined by day length (Farner and Wingfield, 1980; Hau et al., 2008). Thus, the importance of supplemental cues for timing of breeding is likely very different in male and female birds (Farner, 1986; Perfito et al., 2015; Williams, 2012; Wingfield et al., 1997).

Temperature is widely assumed to be an important supplemental cue for timing of egg laying and many correlational field studies have reported a strong, negative relationship between ambient temperature and laying date (warmer temperatures associated with earlier laying) in a wide range of species (Dawson et al., 2001; Visser et al., 2010; Williams, 2012), including European starlings (*Sturnus vulgaris*) (Meijer et al., 1999; Williams et al., 2015). In most avian species, late spring temperatures (March, April, May) that are closer to egg-laying are most tightly associated with laying date, consistent with the idea that short-term variation in temperature is used to fine-tune egg-laying to year-specific local conditions (Brommer et al., 2008; Schaper et al., 2012).



Consequently, many studies examining the relationship between temperature and laying date have restricted their analysis to temperatures closely associated with the breeding seasons of the study species (e.g., Jeong et al., 2020; Shave et al., 2019; Smith et al., 2020; Watts et al., 2019; Wesolowski et al., 2021). However, this is often done with little *a priori* knowledge on the relationship between climate and laying date, leading to arbitrary selections of climate windows (Bailey and van de Pol, 2016; Pol et al., 2016). Some studies widening the selection of potential temperature windows have suggested that temperatures well in advance of laying, such as the previous August (Pearce-Higgins et al., 2010) or mid-winter temperatures (Imlay et al., 2018; Williams et al., 2015), can be predictive of variation in laying date. For example, Williams et al. (2015) previously found that temperatures from 8 January through 22 February were most highly correlated with laying date in European starlings which typically occurs in early to mid April. This would suggest that short-term temperature is not used as a supplemental cue to fine-tune laying, rather it might serve as a long-term cue to time breeding.

Here we re-assess the relationship between ambient temperature, a putative supplemental cue, and timing of egg-laying in European starlings using three complimentary approaches: a) an 'unconstrained', exploratory analysis ('climwin'; Bailey and van de Pol, 2016; Pol et al., 2016), b) using the traditional sliding window approach (Brommer et al., 2008; Husby et al., 2010) to re-analyse temperature-laying date relationships from Williams et al. (2015) but for a longer time period (2002-2021,  $n = 20$  years), and c) analysis of specific, biologically-informed temperature windows previously suggested to influence laying date (see Methods). Additionally, we examine autocorrelations between temperature windows to examine how long-term temperature cues could be used by birds to time breeding to a broad reproductive window. Finally, we explore if the differential or 'mismatch' between early and late season (that is January vs. March) temperatures or the coefficient of variation in temperature are associated with residual variation in laying date to test the hypothesis that variability in temperature, not just mean temperature, might influence laying date. Our overall objective was to examine how, and over what time period, ambient temperature acts as a supplemental cue to fine-tune timing of egg-laying of European starlings to the local environment.

## Methods

### *Breeding data*

We used 20 years of breeding data (2002–2021) from our long-term European starling study at Davistead Farm, Langley, British Columbia, Canada (49°10' N, 122°50' W), which comprises ca 150 nest-boxes mounted on posts around pastures and on farm buildings. Each year, we followed the same basic field protocol: nest-boxes were checked daily from 1 April to determine laying date and clutch size, and all newly laid eggs were weighed ( $\pm 0.001\text{g}$ ) and numbered. In several years (2004, 2005, 2007, 2009), we conducted experiments which involved catching females at clutch completion and/or removing eggs to stimulate laying of replacement clutches (e.g. Love and Williams, 2008). Therefore, we analysed laying date and temperature for all 'first' clutches initiated during a first peak of egg laying in each year. We defined this first peak of laying as the 12-day period from the earliest first nest initiation date in any year based on a mean five egg clutch, two further days for determination of clutch completion and a minimum re-nesting interval after egg removal of 5 days, so that we excluded any potential replacement clutches after early failure of true 'first' nests. All females were banded (US Fish and Wildlife metal bands, plus colour bands; permit # 10646) so we could confirm re-nesting.

### *Temperature data*

Daily temperature data were obtained for the Pitt Meadows weather station, British Columbia (49°12' N, 122°41' W, elevation 5.0 m above sea level) using the Environment Canada online National Climate Data and Information Archive (<http://www.climate.weatheroffice.gc.ca>). Pitt Meadows is less than 20 km from our Davistead Farm study site. Mean monthly temperature at Pitt Meadows was highly correlated with mean monthly and daily temperature at the Cloverdale weather station (20 km south of our study site), and at Vancouver Airport (40km west), and thus provides a good index of variation in regional temperature (Williams et al., 2015). Mean daily temperature was highly correlated with both minimum ( $r^2 = 0.81$ ,  $P < 0.001$ ) and maximum daily temperature ( $r^2 = 0.87$ ,  $P < 0.001$ ), whereas minimum and maximum daily temperatures were only weakly correlated ( $r^2 = 0.45$ ,  $P < 0.001$ ), therefore, we used mean temperature for all subsequent analyses.

## **Statistical analysis**

We first used the R package ‘climwin’ (Bailey and van de Pol, 2016; Pol et al., 2016) to calculate the best climate predictor (temperature) of mean laying date from 2002-2021 by using an objective, non-constrained sliding window approach. This method examines every possible window of climate during a predefined period, with our selection spanning a 365-day interval, then ranks temperature windows using AICc weights, and the model with the lowest  $\Delta\text{AICc}$  output is the temperature window with the highest explanatory effect of climate on laying date. We used an “absolute” window analysis which is used for datasets with little temporal variation in trait expression and used the mean laying date of starlings (9 Apr) as the “reference date”. We quantified the likelihood of obtaining strong model support by chance ( $P_{\Delta\text{AICc}}$ ) (here a linear effect of mean temperature on starling lay dates) by performing the same time-window analysis on  $n = 1,000$  randomizations (*randwin*) of the original climate data. We then ran a weighted window approach (*weightwin*) using Weibull weights which produces a distribution indicating the peak explanatory window while also reflecting gradual/fading effects, here of temperature on laying date. Finally, we used *autowin* to visually examine autocorrelations in temperatures between the single best window and all other temperature windows (Bailey and van de Pol, 2016; Pol et al., 2016).

Second, since the more exploratory approach using climwin also has some drawbacks (e.g. the risk of overfitting and bias, van de Pol et al., 2016) we used the more traditional sliding window approach (Brommer et al., 2008; Husby et al., 2010; Williams et al., 2015). Here, one varies (or slides) the start and end time of a climate window to compare multiple possible windows and select a best window, calculating Pearson’s correlations between the mean annual laying dates and the mean daily temperature. Window size varied from a minimum 10 days for all possible windows between 1 January–31 December. The time period during which the mean temperature provided the highest correlation with the mean laying date was taken to represent the best description of local environmental conditions important for timing of breeding. For example, a window of Julian date 1–10 would represent temperatures for 10 days between 1 and 10 January (furthest from onset of egg laying), whereas a window of 81–90 would represent temperatures for 10 days from 22 to 31 March (just before onset of egg laying). We also calculated the mean  $r^2$  of time periods 6 days before and after what we considered midwinter (Julian 8-53; 8 Jan – 22 Feb) and spring temperature (Julian

60-90; 1 Mar – 31 Mar) to investigate the strength of these time periods as predictors of laying date.

Third, we calculated mean temperatures for specific time windows that have previously been suggested to predict laying date, i.e. we took a confirmatory approach using pre-existing biological knowledge but limiting the number of potential time windows to a few testable hypotheses (e.g. Frederiksen et al. 2014, *sensu* van de Pol et al. 2016). Timing of egg-laying has been linked to both the previous summer (prevaug) and winter (Julian 8-53; 8 Jan – 22 Feb; Williams, 2015) conditions based on predicted effects of temperature on prey (Tipulid) development. Warm or dry summers leave newly deposited eggs and early larval instars in soil vulnerable to desiccation, negatively impacting subsequent spring population size (Pearce-Higgins et al., 2010; Pritchard, 1983) and colder winter temperatures slow or stop the development of third and fourth level instars (Pritchard, 1983). Pre-breeding temperatures two weeks before first egg (ld2wk) are predicted to be important regulators of metabolic processes as increasing temperature is associated with decreasing energy expenditure, and temperatures one week before first egg (ld1wk) are predicted to have a direct effect on physiological processes underpinning yolk development (Williams, 2012). We calculated March temperature (marT) to add an additional measure of “spring” temperatures as well as January (janT) and February (febT) for “mid-winter” temperatures.

Finally, we used Pearson’s correlations to examine the relationship between absolute laying date and the multiple different temperature windows (as described above) and to investigate auto-correlations among different temperature windows. We calculated the residual, temperature-independent laying date as the difference between actual mean laying date and temperature-predicted laying date, using the regression of annual mean laying date and mean temperature for the Julian 2-94 window (2 Jan – 4 Apr). We then compared the mean annual residual, temperature-independent variation with a) the difference between mean January and March temperatures (as an index of slope or increase in temperature, *sensu* Schaper et al. 2012) and b) the coefficient of variation of temperature (cvTa %, as  $sd/meanTa*100$ ) for the Julian 2-94 window. Finally, we ran a linear mixed-effects model using annual mean laying date as the dependent variable and both mean temperature and CV(%) temperature as main effects for the Julian 2-94 window.

## Results

The initial sliding window of 'climwin' showed the single best temperature window associated with laying date opening 208 days before mean laying date (9 Apr) and closing 7 days before laying ( $\Delta AICc = -27.04$ ,  $r^2 = 0.76$ ,  $F_{1,18} = 62.02$ ,  $P < 0.001$ ; Table 1.1). After randomizations, the window within the 95% confidence interval opened 185 days and closed 16 days before laying ( $P_{\Delta AICc} = 0.001$ ; Julian 279 – 83; 6 Oct – 24 Mar; Figure 1.1, 1.2).

Within this broad temperature window, the effect (weight) of temperature on laying date increased as the window opened further from laying date, with the peak effect ranging from approximately 30-75 days before laying (~24 Jan – ~10 Mar) and rapidly decreasing as the window extended beyond 100 days (~31 Dec) ( $r^2 = 0.71$ ,  $P < 0.001$ ,  $F_{1,18} = 48.06$ , Figure 1.2c). This indicates that a broad range of temperatures from mid-January through early-March has a greater effect on timing of egg-laying than temperatures just prior to onset of laying and those preceding January. Additionally, 'climwin' provides a visual representative of autocorrelations between temperatures that best predict laying date and all other temperature windows and there were strong autocorrelations (Figure 1.3) which may explain how a broad window of temperature could be used as a long-term cue to time breeding.

The traditional sliding window approach showed temperature for the period Julian 2-94 (2 Jan – 4 Apr) best predicted laying date ( $r^2 = 0.73$ ,  $P < 0.001$ ,  $F_{1,18} = 51.08$ ; Figure 1.4a). However, a Julian 8-53 temperature 'midwinter' window (8 Jan – 22 Feb) ( $r^2 = 0.58$ ,  $P < 0.001$ ,  $F_{1,18} = 26.9$ ) and other similar 'mid-winter' temperature windows in general were also highly correlated with annual mean laying date (mean  $r^2 = 0.58$ ; range 0.51 – 0.62; for Julian windows 2-47, 3-48, 4-49, 5-50, 6-51, 7-52, 8-53, 9-54, 10-55, 11-56, 12-57, 13-58, 14-59). Conversely, mean January ( $r^2 = 0.23$ ,  $P = 0.02$ ,  $F_{1,18} = 6.66$ ) and February ( $r^2 = 0.32$ ,  $P = 0.01$ ,  $F_{1,18} = 10.03$ ) windows were only moderately correlated with laying, as was March ( $r^2 = 0.4$ ,  $P = 0.002$ ,  $F_{1,18} = 13.42$ ) and the climwin window, Julian 279 – 83 (6 Oct – 24 Mar) ( $r^2 = 0.37$ ,  $P = 0.003$ ,  $F_{1,18} = 12.31$ ).

No immediate pre-breeding temperature window ranked highly in the traditional sliding window analysis (mean  $r^2 = 0.40$ ; range 0.36 – 0.43; Julian windows 54-84, 55-85, 56-86, 57-87, 58-88, 59-89, 60-90, 61-90, 62-90, 63-90, 64-90, 65-90, 66-90), with

laying date being independent of mean temperature of the window 1 -and- 2 weeks before laying, as well as the previous August ( $P > 0.05$  in all cases; Table 1.2). These results are in agreement with those from the 'climwin' analysis in that a broad temperature window was most highly correlated with laying date. As Julian 2-94 (2 Jan – 4 Apr) temperatures were most associated with laying, predicted laying date was calculated using the equation  $(-2.99 \cdot \text{winTa2\_94} + 114.75)$ , and annual residual laying date was then calculated by absolute laying date – predicted laying date. Annual residual laying date varied from -4.04 days in 2013 (a relatively earlier year) to +3.25 in 2018 (a relatively later year; Figure 1.4b).

Climwin analysis suggested strong autocorrelation of temperatures most predictive of laying date with all temperature windows between October to early March (Figure 1.3), as well as strong autocorrelations between other biologically-informed temperature windows (Table 1.2). The top explanatory temperature window for Julian dates 2-94 (2 Jan – 4 Apr) was highly correlated with mean temperature for the Julian 8-53 window (8 Jan – 22 Feb;  $r^2 = 0.85$ ,  $P < 0.001$ ,  $F_{1,18} = 107.8$ ) and moderately correlated with mean January ( $r^2 = 0.37$ ,  $P = 0.003$ ,  $F_{1,18} = 12.1$ ), February ( $r^2 = 0.42$ ,  $P = 0.001$ ,  $F_{1,18} = 14.91$ ), March ( $r^2 = 0.6$ ,  $P < 0.001$ ,  $F_{1,18} = 28.93$ ), and Jul 279-83 temperature windows (6 Oct – 24 Mar;  $r^2 = 0.45$ ,  $P = 0.001$ ,  $F_{1,18} = 16.47$ ). However, mean temperature for the period 2 January – 4 April (Julian 2-94) was independent of mean temperature for the preceding August and the period 1-week before laying ( $P > 0.05$ ), and was only weakly correlated with mean temperature for the period 2-weeks before egg-laying ( $r^2 = 0.19$ ,  $P = 0.03$ ,  $F_{1,18} = 5.49$ ; Table 1.2).

Annual residual, temperature-independent variation in laying date varied from 4.04 days earlier than the temperature-predicted date (2013) to 3.25 days later than the temperature-predicted date (2018; Figure 1.4b). However, residual variation in laying date was independent of the January to March temperature differential ( $r^2 = 0.05$ ,  $P > 0.95$ ,  $F_{1,18} = 0.003$ ; Figure 1.5a) and the coefficient of variation of temperature for the Julian 2-94 window (2 Jan – 4 April;  $r^2 = 0.06$ ,  $P > 0.90$ ,  $F_{1,18} = 0.007$ ,  $P > 0.90$ ; Figure 1.5b; Table 1.3) suggesting that temperature differential is not associated with timing of egg-laying of European starlings.

## Discussion

Here, we re-assessed the relationship between ambient temperature, a putative “supplemental cue”, and timing of egg-laying in European starlings using three complimentary approaches: an ‘unconstrained’ analysis (climwin), a more traditional sliding window analysis, and analysis of specific, biologically-informed temperature windows. Climwin identified a very broad window of ‘predictive’ temperatures from 6 Oct – 24 Mar (169 days), but within this model the period with most influential daily temperatures spanned approximately 24 Jan – 10 Mar. The traditional sliding window analysis, with an unconstrained selection interval, also identified a relatively broad temperature window (2 Jan – 4 Apr, 92 days). There was no support for an influence of temperatures during a shorter period immediately before breeding (cf. many previous studies; see below). Similarly, there was no support for an influence of daily temperature predicted by effects on Tipulid prey development (previous August temperatures, Pearce-Higgins et al., 2010), or through general effects on pre-laying energy expenditure or reproductive physiology of laying females. However, we confirmed that mid-winter temperatures alone (Julian date 8 – 53, Williams et al., 2015) were correlated with variation in laying date ( $r^2 = 0.58$ ). Thus, our study suggests that temperature provides a relatively long-term cue for timing of egg-laying in European starlings, with temperatures from mid-winter (January/February) to the immediate pre-breeding period (March) being most informative of variation in laying date.

Taking an unconstrained, objective and exploratory approach ‘climwin’ (Bailey and van de Pol, 2016; Pol et al., 2016) can help to distinguish potentially co-occurring or co-varying effects of weather signals acting at different stages of an organism’s life cycle. Users can define a baseline model allowing for analysis with a variety of error distributions, the inclusion of multiple covariates, the use of mixed-effects modeling, and different types of regression models (Pol et al., 2016). While an increasing number of studies have used ‘climwin’ to analyse climate effects on fecundity and survival (e.g., Mundinger et al., 2021; Sloan et al., 2022; Stapelfeldt et al., 2022) as far as we are aware few studies have looked at timing of laying. In their original paper using ‘climwin’, Bailey and van de Pol (2016) found that a relatively broad temperature window (11 Feb – 23 Apr) was most predictive of egg-laying date in common chaffinch (*Fringilla coelebs*) though there was a decaying influence of temperature when moving further from the

onset of laying. In our study, 'climwin' analysis suggested a very long, likely biologically unrealistic, window (~6 months) though within this the most influential temperatures were more similar to Pol et al. (2016) results with the most influential temperatures spanning three months (Jan/Mar) and a decaying influence of temperature extending beyond this window (Figure 1.2c). Similarly, in another study using 'climwin', Smith et al. (2020) reported a long-range effect of temperatures south of the breeding ground on laying date of field sparrows (*Spizella pusilla*) (4 Feb – 23 Apr) though temperatures on the breeding grounds, close to laying, (23 Apr – May 22) were also associated with laying date (Smith et al., 2020). This may reflect autocorrelations between temperatures (see below) experienced at different stages of the annual cycle, as birds must be energetically and physiologically prepared for costs associated with sequential life-stages (migration, breeding, and moulting) (Saino et al., 2004; Selonen et al., 2021). Importantly, this preparation overlaps with other temperature-dependent biological processes (i.e., vegetation phenology, resource availability and quality, growth and development of prey, gonadal development) that influence avian fitness (Marrot et al., 2018; Saino et al., 2004), therefore birds may use signals from co-occurring temperature-dependent processes to predict environmental conditions during the breeding season. Thus, there might be a suite of temperature-dependent changes leading to a bird's decision to lay, supporting the view that temperature provides an integrated long-term cue in contrast to the generally accepted view that temperatures alone fine-tune timing of laying.

Similar to 'climwin', our traditional sliding window analysis also indicated a relatively long temperature window most predictive of laying (~3 months, 2 Jan – 4 Apr). Studies using more broad temperature window selections have found differing ranges of influential temperature periods: Williams et al. (2015) reported a window of 8 Jan – 22 Feb ('midwinter') and Huchler et al. (2020) reported a window from 3.5 – 5 weeks before laying. Additionally, in one population of great tits (*Parus major*), Husby et al. (2010) reported a temperature window of 15 Feb – 25 Apr, though, interestingly, they found that a second population of the same species had a substantially shorter time window that was closer to laying (~1 month; 13 Mar – 20 Apr). Other studies have found support for long-term temperatures having an indirect effect on reproductive timing, possibly through interactions with environmental conditions at nonbreeding grounds (i.e., influencing the body condition and time it takes to acquire energy essential for departing overwintering grounds, the arrival time on breeding grounds, length of time it takes to reach



reproductive readiness (Carey, 2009; Imlay et al., 2018; Saino et al., 2004; Selonen et al., 2021; Williams, 2012)) or cues from changes in daylength associated with migratory and breeding preparations (Carey, 2009). Weather on the wintering grounds, along the migration route, and at the breeding site have different effects on reproductive parameters, however the temperature window most relevant to reproductive timing remains unknown (Wiebe and Gerstmar, 2010).

Temperature-dependent processes provide important resources for birds (i.e., seed germination, leaf production, flowering, insect development), many of which are associated with winter climatic conditions, which can cause significant carry-over effects to reproductive timing and success (Carey, 2009; Saino et al., 2004; Selonen et al., 2021; Visser et al., 2009). For example, Saino et al. (2004) found that primary productivity, or vegetation biomass, in the winter (Dec – Feb) affected different life history processes related to reproductive success in barn swallows (*Hirundo rustica*), such as wing and tail length during prenuptial moult. Additionally, low vegetative biomass on winter grounds resulted in delayed laying and lower fledgling success in comparison to years of high quality (Saino et al., 2004). Thus, it is plausible that temperatures well in advance of laying can affect timing of breeding. Despite this possibility of ‘carry-over effects’ from conditions on nonbreeding grounds on reproductive timing (Selonen et al., 2021), previous analyses have often selected limited, arbitrary temperature windows associated with breeding (e.g. late Spring) which may explain why it is generally accepted that temperatures closest to onset of egg-laying, or “spring” temperatures, are most predictive of laying date (e.g. Brommer et al., 2008; Burant et al., 2022; Meijer et al., 1999). However, after removing restrictions on temperature constraints from the traditional sliding window analysis, our study found that a broad temperature window, January – March, was most predictive of laying. We found little support for the key idea (Farner and Wingfield, 1980; Farner, 1986; Visser, 2010) that immediate pre-breeding temperatures are used as supplemental cues to fine-tune timing of egg-laying to local weather conditions.

We found no support in our study for other biologically-informed windows previously suggested to influence timing of egg-laying, including temperatures in the previous August (prey development), two weeks before laying (metabolic processes), or within one week of laying (final gonadal maturation). Temperatures one week prior to onset of egg-laying may directly initiate processes needed to reach reproductive maturity

through costs associated with gonadal development and egg production. Initiation of vitellogenesis to ovulation of the first egg can happen within one week in many avian species, and low temperatures are suggested to increase these costs, directly influencing timing of egg-laying (Visser et al., 2009). Temperatures two weeks prior to onset of laying likely have a direct effect on metabolic processes (i.e., acquiring energy needed for the cost of egg production, rate of ovarian development, female energy expenditure; Williams, 2012). Although plausible, we, again, found no support for temperature-dependent physiological or metabolic processes directly effecting timing of egg-laying. Pearce-Higgins et al. (2010) found that previous August temperatures were associated with reproductive success in golden plovers (*Pluvialis apricaria*) through its effect on Tipulid abundance and development: the years with the warmest temperatures in the previous August saw crane fly abundance decrease to <5% of the maximum number observed. Tipulids, starlings main prey choice, are thought to be particularly vulnerable to summer temperatures in their newly-deposited egg state, as warm summers and drought increase the likelihood of desiccation which may allow for predictable variations in their abundance and quality the following spring (Pearce-Higgins et al., 2010). While we found no support for an influence of previous August temperatures on timing of egg-laying in European starlings, our results also support the general idea that long-term temperature cues could provide indirect cues used to time laying to a broad reproductive window.

Analysis using climwin showed strong autocorrelation of temperatures over a broad range of time periods (6 Oct – 24 Mar) including both short-term temperature windows just prior to laying and long-term temperatures further from the breeding season (positive correlation represented by beige on the climwin map; Figure 1.3). Similar to our climwin results, we found that mean midwinter, January, February, and March temperatures were all strongly correlated with temperatures in our most predictive window (~3 months, 2 Jan – 4 Apr), though there were no correlations with temperatures the previous August or one -and- two weeks before laying. This supports the idea that autocorrelations of temperatures may allow birds to predict upcoming favourable conditions (Visser et al., 2009). Selonen et al. (2021) found in a population of pied flycatchers (*Ficedula hypoleuca*), that temperatures even hundreds of kilometers away were autocorrelated with those at breeding locations, and nonbreeding temperatures had a greater association with reproduction than breeding ground temperatures. This

population overwinters in areas with relatively constant winter temperatures, which may allow for birds to predict environmental conditions during the breeding season (Selonen et al., 2021). Similarly, when examining the effect of warming (i.e. increasing) temperatures on timing of egg-laying in a non-migratory population of blue tits (*Cyanistes caeruleus*), Marrot et al. (2018) found that winter temperatures (Dec – Feb) were more stable than those closer to the period of laying. Stable environmental conditions during the winter and its autocorrelation with temperatures during the breeding season, as well as co-occurring temperature-dependent processes causing carry-over effects, may allow for birds to use long-term temperature cues to reliably predict a broad reproductive window conducive to successful breeding.

In summary, we found that a long-term temperature cue, spanning January through March, best predicted onset of egg-laying in a local population of European starlings with a significant influence of mid-winter temperatures (as shown in Williams et al., 2015). However, there was no support for short-term temperature cues within ~1 month of laying being associated with variation in laying date. This contrasts markedly with the widely held idea that immediate, pre-breeding, “spring temperatures” best predict short-term variation in laying allowing birds to fine-tune timing of breeding to local conditions around the time of egg-laying. Our study suggests, instead, that a broad range of temperatures, including both winter and spring temperatures, may predict variation in laying date quite well ( $r^2 \sim 60-70\%$ ), so mechanisms that allow integration of long-term temperature information must exist in birds (*sensu* Caro et al. 2013). However, given the highly synchronous breeding of European starlings, considerable residual temperature-independent variation in laying date still exists in our study system: starlings initiated laying from four days earlier to three days later than that predicted by temperature alone. This is not explained by other components of temperature cues (i.e. deviation from early January and later March, variation in temperature (CV%)) which leaves the question of whether this residual variation is related to some other supplemental cue, e.g. food or social factors, and the latter hypothesis will be explored in Chapter 2.

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## Tables

**Table 1.1** Output of 'slidingwin' showing the best model ( $\Delta AICc$ ) reflecting all fitted climate windows using an "absolute" level analysis (little temporal variation in the response variable) on mean and slopes using linear and quadratic functions of the 'single best' temperature window predicting laying date. WindowOpen represents the number of days before laying that the climate window opened while WindowClose indicates the number of days before laying that the climate window closed.

Response	Climate	Type	Stat	Func	DeltaAICc	WindowOpen	WindowClose
xLD	Temp	absolute	mean	lin	-27.04	208	7
	Temp		slope		-11.01	344	340
	Temp		mean	quad	-27.32	141	7
	Temp		slope		-18.04	162	145

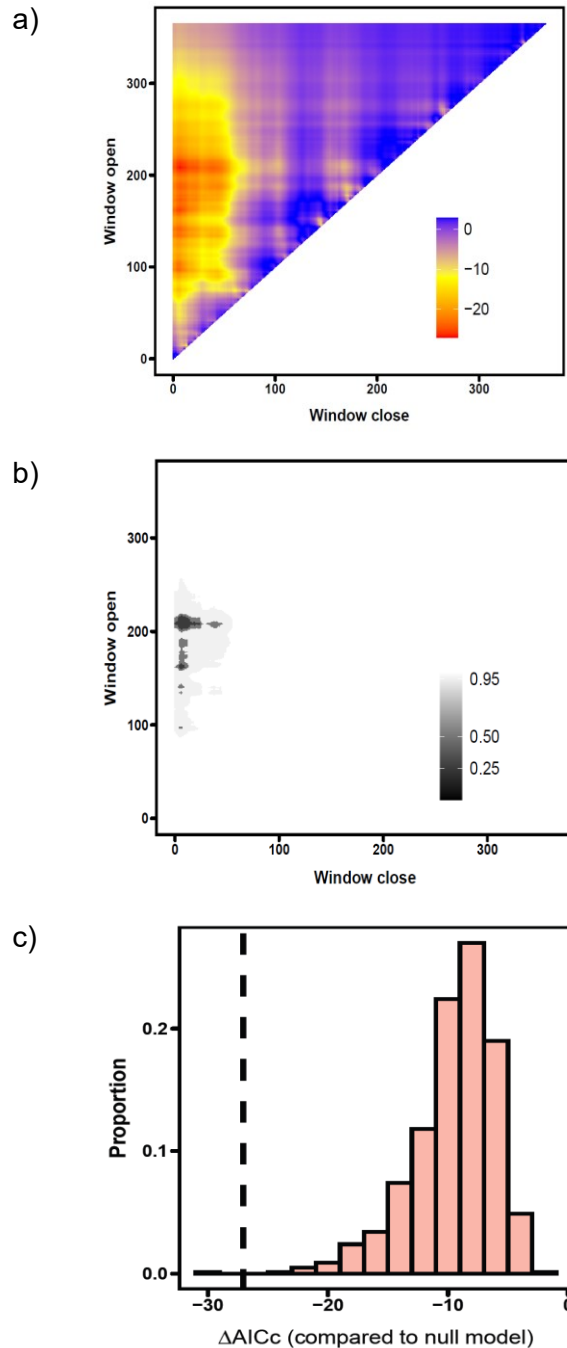
**Table 1.2 Relationships between actual laying date (xdate), predicted laying dates based on Julian 2-94 temperature window (winTa2\_94), and mean temperatures for different periods (previous August, Julian 8-53, Jan, Feb, Mar, 1 and 2 weeks before first egg-laying and Julian 279-83 (win16\_185)). Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Number in parentheses indicates sample size.**

	xdate	winTa2_94	prevaug	win8_53	janT	febT	marT	ld1wk	ld2wk	win16_185
xdate	1	<b>-0.86***</b> (20)	-0.24 (20)	<b>-0.77***</b> (20)	<b>-0.52*</b> (20)	<b>-0.6**</b> (20)	<b>-0.65***</b> (20)	-0.13 (20)	-0.37 (20)	<b>-0.64***</b> (20)
winTa2_94		1	0.32 (20)	<b>0.93***</b> (20)	<b>0.63***</b> (20)	<b>0.67***</b> (20)	<b>0.79***</b> (20)	0.12 (20)	0.48 (20)	<b>0.69***</b> (20)
prevaug			1	0.25 (20)	0.26 (20)	-0.02 (20)	0.4 (20)	0.41 (20)	0.31 (20)	<b>0.57**</b> (20)
win8_53				1	<b>0.65***</b> (20)	<b>0.69***</b> (20)	<b>0.61***</b> (20)	-0.01 (20)	0.43 (20)	<b>0.59**</b> (20)
janT					1	0.06 (20)	0.27 (20)	0.04 (20)	0.42 (20)	0.35 (20)
febT						1	0.38 (20)	-0.18 (20)	-0.07 (20)	0.41 (20)
marT							1	0.3 (20)	<b>0.61***</b> (20)	<b>0.69***</b> (20)
ld1wk								1	<b>0.47*</b> (20)	0.16 (20)
ld2wk									1	<b>0.5*</b> (20)
win16_185										1

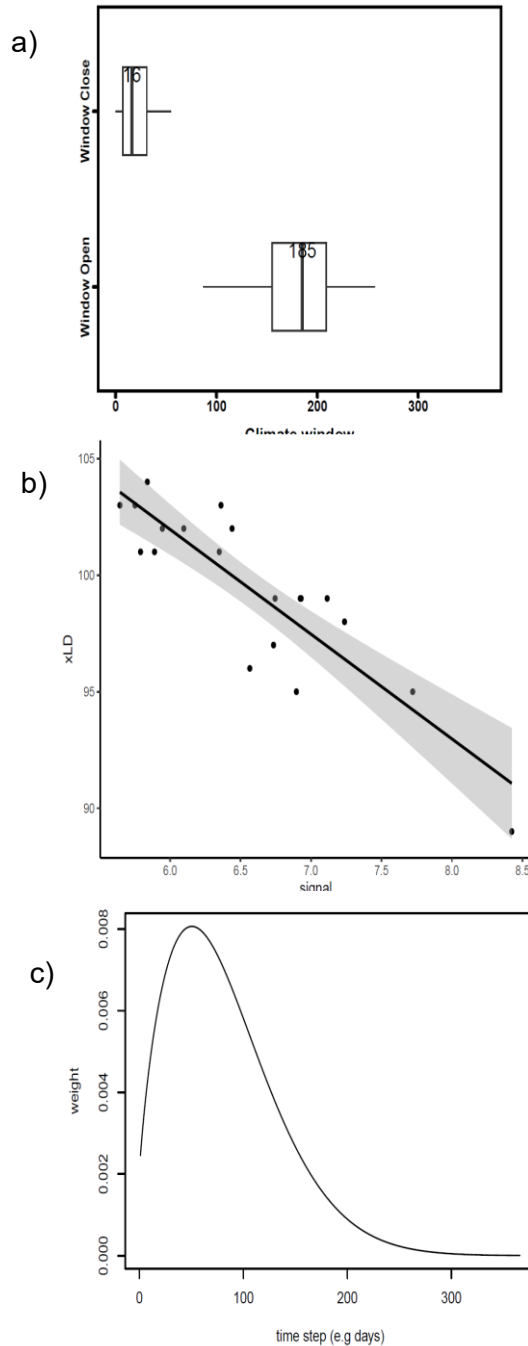
**Table 1.3 Relationships between actual laying date (xdate) and deviation from predicted laying dates (devxLD) based on Julian 2-94 temperature windows, and mean coefficient of variation of temperatures for different temperature periods (Julian 2-94, Julian 8-53, Jan, Feb, Mar, Julian 279-83 (cvTa16\_185)) and the temperature differential between Jan and Mar across years. Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Number in parentheses indicates sample size.**

	xdate	devxLD	cvTa2_94	cvTa8_53	cvTa_janT	cvTa_febT	cvTa_marT	cvTa16_185	devjan_mar
xdate	1	<b>0.54**</b> (20)	<b>0.66***</b> (20)	<b>0.59**</b> (20)	<b>0.47*</b> (20)	0.06 (20)	0.29 (20)	<b>0.53**</b> (20)	-0.03 (20)
devxLD		1	-0.03 (20)	-0.05 (20)	0.02 (20)	-0.17 (20)	-0.26 (20)	0.08 (20)	-0.01 (20)
cvTa2_94			1	<b>0.87***</b> (20)	<b>0.72***</b> (20)	0.43 (20)	0.43 (20)	<b>0.46*</b> (20)	0.35 (20)
cvTa8_53				1	<b>0.56**</b> (20)	<b>0.52*</b> (20)	0.27 (20)	0.3 (20)	0.24 (20)
cvTa_janT					1	-0.18 (20)	0.05 (20)	0.24 (20)	<b>0.63***</b> (20)
cvTa_febT						1	0.28 (20)	0.15 (20)	-0.1 (20)
cvTa_marT							1	0.33 (20)	-0.42 (20)
cvTa16_185								1	0.17 (20)
devjan_mar									1

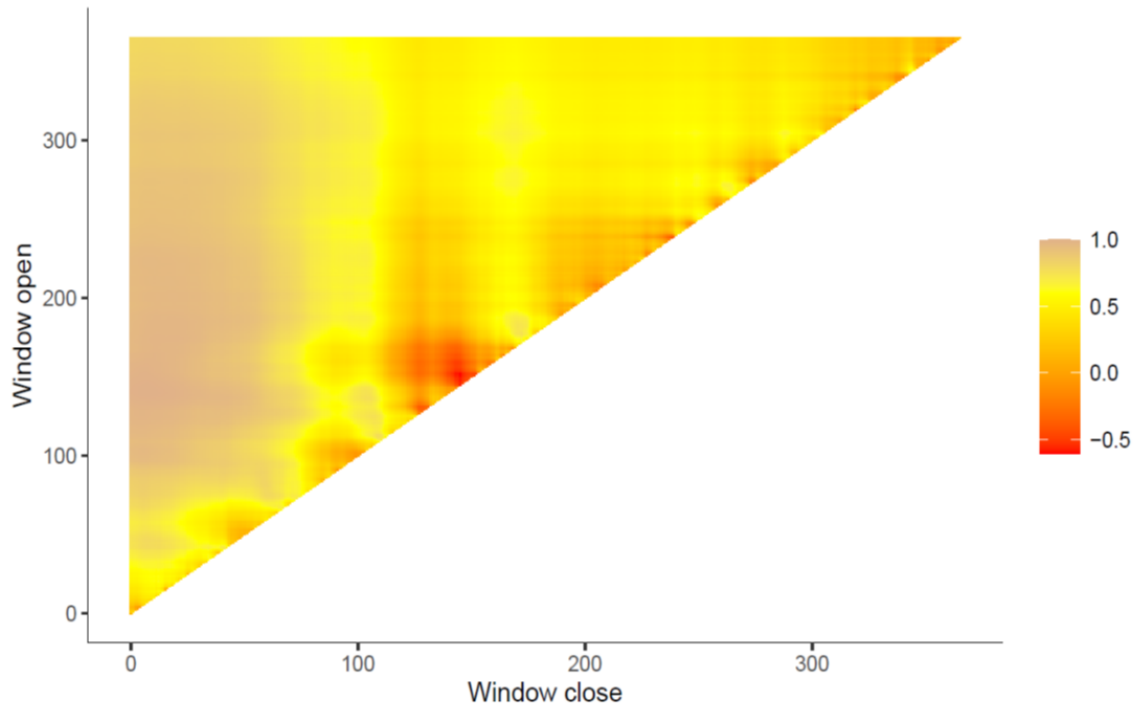
## Figures



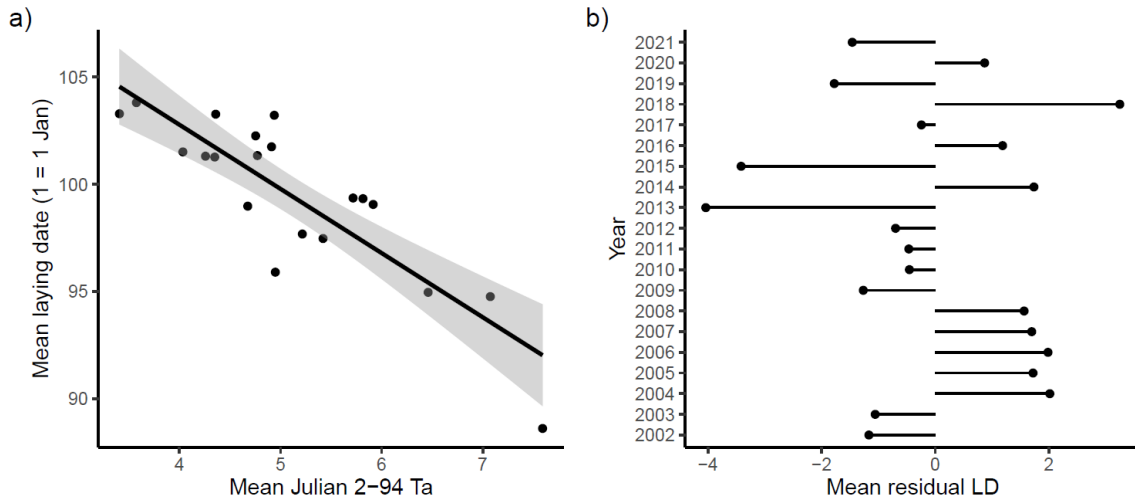
**Figure 1.1** Analysis testing the relationship between mean temperature and laying date in the European starling (*Sturnus vulgaris*) using a reference day of April 9<sup>th</sup> a) Heat map of the model support ( $\Delta AICc$ ) for all fitted climate windows (Julian date). Models with the lowest  $\Delta AICc$  (red) are the best supported, b) 95%, 50%, and 25% confidence sets for all fitted climate models, and c) distribution of  $\Delta AICc$  indicating the climate-laying date relationship is unlikely due to chance. Window open represents the number of days before the reference date that the climate window opened while window close indicates the number of days before the reference date that the climate window closed.



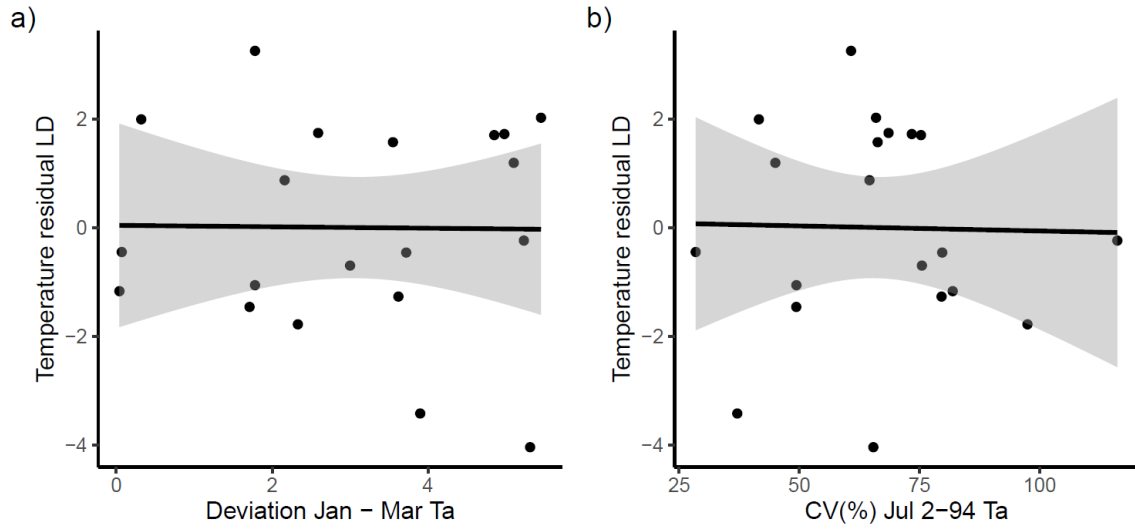
**Figure 1.2 Results of climwin showing a) the opening and closing points of time windows (Julian days) best supported by the data (within 95% model confidence set) after 1,000 randomisations (*randwin*) opening 185 days before laying (window open; 6 Oct) and closing 16 days before laying (window close; 24 Mar) (Julian 279 – 83), b) a negative correlation between mean laying date of the population (y-axis; xLD) and temperature (x-axis; signal) and c) weighted window analysis (*weightwin*) illustrating the ‘weight’ of the temperature window ~30 – 75 days before laying (~24 Jan - ~10 Mar) with temperatures beyond this range having a lesser effect on laying date. Time step on y-axis indicates the day of year and weight on the x-axis indicates weight or strength of the climate signal on laying date.**



**Figure 1.3** climwin analysis (*autowin*) showing the strength of correlation between the mean temperature during each time window and the mean temperature during the best supported time window. Window open represents the number of days before laying that the climate window opened while window close indicates the number of days before laying that the climate window closed. Beige indicates positive correlations between a broad range of temperatures.



**Figure 1.4** The traditional sliding window analysis results showing the relationship between a) annual mean laying date and annual mean Julian 2-94 (2 Jan – 4 Apr) temperatures and b) annual mean temperature-predicted residual laying date. Data from 2002 – 2021.



**Figure 1.5** The relationship between mean temperature-independent laying date Julian 2-94 (2 Jan – 4 Apr) against a) temperature differential between Jan – Mar temperatures and b) mean coefficient of variation (CV(%) of Julian 2-94 temperatures for years 2002-2021.



## Chapter 2. Female social networks and timing of egg-laying in European starlings (*Sturnus vulgaris*)

### Abstract

Research on social factors as “supplemental cues” for timing of egg-laying in birds has mainly focused on the roles of male displays (courtship, singing) despite the fact that females are the sex that controls the egg-laying decision. Female-female interactions (social networks) may benefit females by providing shared information on environmental conditions, matching food availability with provisioning, group foraging to locate food, and stimulation of ovarian development and reproductive behavior. This might then allow for fine-tuning of laying date, increasing breeding synchrony, and enhancing reproductive success. The limited research on female social interactions has mainly focused on the relationship between measures of sociality and laying synchrony or breeding success and there is only limited, and inconsistent, research examining how spatial structure and other social network measures relates to laying date. Here, we analysed the relationship between social network structure and variation in timing of egg-laying in highly social, semi-colonial European starlings (*Sturnus vulgaris*) at a population and individual level to determine if, and how, five female social network metrics (neighbour distances, residency, female -and- network familiarity, and synchrony) might act as supplemental cues to fine-tune timing of egg-laying. We found that individual residual, temperature-independent laying date was associated with overall network familiarity and nearest neighbour distances in a “linear” habitat. This suggests that some components of female-female social network structure could act as a supplemental cue for timing of egg-laying.

**Keywords:** Birds; Females; Social networks; Supplemental cues

### Introduction

Timing of breeding by female birds, specifically timing of egg-laying, has important consequences for fitness: birds must time peak demand for food from chicks in the nest to seasonal peaks of food availability, which are often of limited duration (Williams, 2012). However, physiological and behavioural preparations for breeding can

take weeks to months and birds use information from various environmental cues or “proximate” factors to predict time of onset of laying (Farner and Wingfield, 1980; Visser et al., 2009, 2012; Wingfield et al., 1997). In both sexes, photoperiod provides “initial predictive” information that initiates seasonal reproductive development (Dawson, 2008; Dawson et al., 2001; Sharp, 1996) with a “switch” on of upstream components of the reproductive axis (at the level of the hypothalamus and pituitary). This is thought to be sufficient for males to reach full reproductive maturity (testis development, spermatogenesis). However, in females the final stages of gonadal maturation occur just prior to onset of egg-laying and it is now more widely accepted that female gonadal maturation is regulated mostly downstream in the hypothalamic-pituitary-gonadal axis at the level of the ovary and liver (Ball and Ketterson, 2008; Perfito et al., 2015; Verhagen et al., 2019; Williams, 2012). Furthermore, this final step of gonadal maturation in females is thought to involve integration of a broader range of environmental information from non-photic “supplemental cues” (temperature, food, social factors) to fine-tune timing of egg-laying to local conditions within the broader reproductive window determined by day length (Farner and Wingfield, 1980; Hau et al., 2008). Temperature is thought to be a strong determinant of egg-laying date in a variety of avian species, with increasing temperatures often associated with earlier laying (Brommer et al., 2008; Schaper et al., 2012; Chapter 1). However, most studies examining the effects of temperature report considerable residual (temperature-independent) variation with temperature alone explaining ~70% of overall variation in egg-laying dates (Husby et al., 2010; Williams et al., 2015; Chapter 1). This suggests that other supplemental cues such as food or social factors might also contribute to variation in timing of egg-laying.

In relation to timing of gonadal development and egg-laying in females, our knowledge of non-photic supplemental cues, other than temperature, is patchy (Chmura et al., 2020; Perfito et al., 2015; Visser et al., 2010). Helm et al. (2006) suggested that females may adjust laying based on “public information” (Helm et al., 2006) on environmental conditions (i.e., food availability, quality of nesting sites, territory distribution, predator awareness) from conspecifics (also see, Immer et al., 2021). This may be beneficial for individual females as they can incorporate the experiences from multiple females into ‘decision-making’, increasing the reliability of information (Brandl et al., 2019; Evans et al., 2009; Immer et al., 2021). However, most studies of social cues for timing of breeding have focused on male displays (courtship, singing) despite the fact

that females are the sex that controls the egg-laying decision (Williams, 2012). Female-female displays have been shown to stimulate ovarian development (Ball and Ketterson, 2008; Cheng et al., 1998; Immer et al., 2021; Waas et al., 2005), potentially through sensory receptors in the visual and auditory system that might be integrated into the hypothalamic-pituitary-gonadal axis generating an endocrine response (Ball and Ketterson, 2008). Female-female social interactions could be positive, e.g., stimulating ovarian development and increasing reproductive synchrony and success (i.e., matching food availability with provisioning, group foraging, predator dilution), or negative, disrupting breeding synchrony and success due to stress, territorial aggression, isolation, or unreliable information (Darling, 1938; Evans et al., 2009; Helm et al., 2006; Immer et al., 2021).

The limited research on female social interactions has mainly focused on the relationship between measures of sociality and laying synchrony or breeding success, not timing of egg-laying *per se*. This work, mainly in (semi) colonial nesting species, has considered measures of spatial structure at the breeding site (Birkhead, 1977; Yom-Tov, 1975), nearest neighbour distances (Evans et al., 2009; Mougín et al., 2001), familiarity with the environment (Westneat, 1992), female-female familiarity (Riehl and Strong, 2018), and social stimulation via vocalisations (Cheng et al., 1998; Waas et al., 2005). Studies investigating spatial structure of breeding locations (“dense” vs “sparse” nesting, neighbour distances) suggests that females nesting with denser spatial structure, and with shorter nearest neighbour distances, have greater breeding synchrony and increased breeding success than those females nesting at greater distances within the cluster (Birkhead, 1977; Evans et al., 2009; Mougín et al., 2001; Yom-Tov, 1975). In contrast to synchrony of laying, there is only limited, and inconsistent, research examining how spatial structure relates to laying date; for example, Westneat (1992) found that residency, the number of years of prior residency by the same female, was strongly associated with first egg date. In contrast, Yom-Tov (1975) found no significant difference in laying dates between clumped and dispersed nests, though synchrony was negatively related to inter-nest distances. Thus, despite social interactions being thought of as supplemental cues to fine-tune laying for over eighty years (Darling, 1938), relatively little progress has been made in understanding how social dynamics relate to reproductive timing (cf. synchrony). Importantly, given the strong influence of temperature there appear to have been no studies examining the relationship between

social structure and temperature-independent laying (i.e., residual variation). To our knowledge, ours is the first study investigating how social factors might contribute to deviation from the temperature-predicted laying date.

Here, we analyse the relationship between social network structure and variation in timing of egg-laying in European starlings (*Sturnus vulgaris*) by examining if a) annual, population-level and b) individual variation in five female social network metrics (nearest neighbour distances, residency, female -and- network familiarity, and synchrony; see Methods) are associated with annual mean laying date and temperature-predicted residual laying date (Chapter 1) as well as autocorrelations in social metrics, and c) if nesting location or overall spatial distribution of nesting locations ('linear' vs 'clumped') is associated with variation in timing of egg-laying, and with social network structure. Our overall objective was to determine if, and how, five female social network metrics might act as a supplemental cue to fine-tune timing of egg-laying to the local environment.

## Methods

### ***Breeding data***

We used 20 years of breeding data (2002–2021) from our long-term European starling study at Davistead Farm, Langley, British Columbia, Canada (49°10' N, 122°50' W). This site comprises *ca* 150 nest-boxes mounted on posts around pastures and on farm buildings categorised into three locations and, importantly for this study, two different nest-box distributions 'linear' and 'clumped'. Nest-boxes in the 'linear' habitat have a continuous alignment along a fence line whereas boxes in the 'clumped' habitat are in a dense, clustered arrangement mainly on barns. Main Field (MF) had a linear arrangement of nest-boxes and Old Barn (OB) and New Barn (NB) had nest-boxes in a clumped or clustered arrangement (Figure 2.1). Each year, we followed the same basic field protocol: nest-boxes were checked daily from 1 April to determine laying date and clutch size, and all newly laid eggs were weighed ( $\pm 0.001$  g) and numbered. Therefore, we restricted analysis of laying date, temperature, and sociality to all 'first' clutches initiated during a first peak of egg-laying in each year. We defined this first peak of laying as the 12-day period from the earliest first nest initiation date in any year based on a mean five egg clutch, two further days for determination of clutch completion and a minimum re-nesting interval after egg removal of 5 days (Fowler and Williams, 2017;

Williams et al., 2015), so that we excluded any potential replacement clutches after early failure of true 'first' nests. All females were banded (US Fish and Wildlife metal bands, plus year-specific colour bands; permit # 10646) so we could confirm re-nesting.

### **Female social networks**

Global Positioning System (GPS) coordinates of ~150 European starling nest-box locations were collected in October 2019 using a GPS (Garmin eTrex® 30) unit at Davistead Farm, which were then mapped using ArcGIS online (Esri©). The ArcMap (<https://arcg.is/0Dqez51>) has layers for each nesting season from 2011-2021 and data including nest-box number, female band number, and laying date (format: laydate, box#, band#). This was used to measure distances (m) to the nearest 12 nest-boxes whether occupied or not (distance key) and seven nearest neighbour distances for occupied nest-boxes in meters. To confirm the ArcGIS estimated distance measurements, actual inter-nest distances were measured in the field for  $n = 39$  nest-box distances to 'ground-truth' the ArcGIS system. ArcGIS estimated distances were highly positively correlated with measured distances ( $r^2 = 0.998$ ,  $b = 1.00$ ; Figure 2.2). Therefore, all distance data used in subsequent analysis was calculated using the created ArcMap.

We generated five different measures to characterise the social networks of all focal females, i.e. known or banded females at known nest-box locations, using data from 2011-2021, where social network metrics were only calculated when observed in consecutive years (data for 2011 were only used to generate values for returning females in 2012):

a) **Nearest neighbour distances** (m): we initially defined a focal female's social network as the mean distance to her first to seventh nearest neighbours (i.e. occupied boxes). There was strong autocorrelation among all inter-nest box distances from the 1<sup>st</sup> to 7<sup>th</sup> nest, as well as mean inter-nest box differences for neighbours 1-3 and 1-7 (Table 2.1). In addition, there was a significant year\*location interaction for the nearest nest ( $P < 0.001$ ,  $F_{20} = 2.89$ ), the mean distance to the three nearest occupied nests ( $P < 0.001$ ,  $F_{20} = 2.8$ ) and mean distance to the seven nearest occupied nests ( $P < 0.001$ ,  $F_{20} = 5.14$ ). Thus, since all inter-nest distance measures were highly correlated, we used mean nearest neighbor distances for the 1<sup>st</sup> to 7<sup>th</sup> nests in subsequent analysis since this captures a larger social network.

b) **Residency**: the number of sequential years a female nested in the same nest-box (1 indicating the year of first detected use). Residency over three years was combined (3+) to account for the small sample size (Residency = 1 year, n = 306; residency = 2 years, n = 54; residency = 3+ years, n = 23).

c) **Female familiarity**: the proportion of females that a focal female had nested with in the previous year within her social network of the seven closest nesting females, i.e. the number of familiar neighbors a female has. A familiarity score was calculated by dividing the number of known females by 7. Familiarity = 1 means the focal female nested with all seven of the same female neighbours in the previous year; familiarity = 0.14 means the focal female nested with only one of her neighbours from the previous year.

d) **Network familiarity**: the proportion of females that nested in or around a focal box the previous year. A familiarity score was calculated by dividing the number of returning females by 8. Familiarity = 1 means the same 8 females returned to the same nesting area the following year and were among the seven nesting neighbours closest to the focal box (8 including the female in the focal box). Familiarity = 0.125 means only one female from the previous year nested within the same network.

e) **Synchrony**: the variance (coefficient of variation) of laying date for the seven-female social network of each focal female, calculated as the standard deviation divided by the mean laying date of the network. A higher synchrony score or CV (%) indicates less synchrony in onset of laying and a lower synchrony score indicates a high level of synchrony in onset of laying.

### ***Statistical analyses***

#### Annual and location variation in laying date and social network metrics

All analysis were conducted in R version 4.1.1 (R Core Team, 2021). First, we asked whether there was significant annual (yearly) variation and nesting location variation in a) mean absolute laying date (clutch initiation date based on Julian calendar) of all females, b) and mean temperature ( $T_a$ )-independent laying (the difference between mean absolute laying date in each year and the predicted laying date based on the Julian 2-94 temperature window (2 Jan – 4 Apr; see Chapter 1)). Nesting location

refers to the two “clumped” locations (Old Barn and New Barn) and the “linear” nesting location (Main Field; see Figure 1). We then asked if there was significant annual (yearly) variation and nesting location variation for our five social network metrics (neighbour distances, residency, female -and- network familiarity, synchrony). For these analyses we used linear mixed-effect models ('lmer') with absolute and temperature-independent laying date or social network metrics as the dependent variable, year, nesting location and their interaction as main effects and either band or box as random factors. If the year\*location interaction was significant we analysed data for each year separately with nesting location as the main effect and calculated marginal means ('emmeans') and pairwise contrast ('contrast') where nesting location was significant. Next, we ran a Pearson's correlation matrix to examine the relationship between variation in mean absolute laying date and temperature-independent laying date with means for each of the five social network metrics at the level of year (n = 10) and year\*location (n = 30).

#### Individual variation in laying date and social network metrics

We calculated individual variation in timing of egg-laying as, a) residual laying date: the difference between an individual's actual laying date each year and the annual mean laying date for that year, i.e., were individuals relatively 'early' or 'later' laying; and b) residual temperature-predicted laying date: the difference between an individual's actual laying date and the mean laying date predicted for that year using a Julian date 2-94 temperature window (2 Jan – 4 Apr; Chapter 1). Individual variation in laying date was then analysed with the five social network measures (neighbour distances, residency, female -and- network familiarity, and synchrony) using linear mixed-effect models ('lmer') with residual or temperature-predicted residual laying date as the dependent variable, each social network metric, nesting location (MF, OB, NB) and the interaction as main effects, with either band or box as random factors. If the social trait\*location interaction was significant we analysed data for each location separately with the differing social network metric as the main effect, and calculated marginal means ('emmeans') and pairwise contrast ('contrast'). Next, we ran a Pearson's correlation matrix to examine the relationship between residual and temperature-predicted residual laying date and correlations among social network metrics at an individual level.

## Results

### Annual and location variation in laying date and social network metrics

There was a significant year\*location interaction for laying date and temperature-independent laying date ( $P < 0.01$  in both cases), as well as for each of the five social network metrics ( $P < 0.04$  in all cases; Table 2.2). We therefore analysed location differences (between linear (MF) and the two clumped (OB and NB) nesting areas) for each year separately. Laying date varied with nesting location in only 1/11 years (2013), with laying being relatively later in OB (Julian 96.9) compared to MF (Jul 95.2) and NB (Jul 95.8) ( $P = 0.03$ ,  $F_{2,76} = 3.61$ ; Figure 2.3). Similarly, temperature-independent laying date varied with location in only 1/11 years (2013), with mean laying date 3.09 days earlier than predicted by temperature at OB, 4.18 days earlier than predicted at NB, and 4.68 days earlier than predicted at MF ( $P = 0.03$ ,  $F_{2,76} = 3.61$ ).

For social network metrics, there were strong and consistent differences in nearest nest distance in relation to nesting location in 11/11 years ( $P < 0.001$ , in all cases; Figure 2.4). Overall, mean distance to the seven nearest nests was almost three times greater in MF ( $44.6 \pm 20.9$  m,  $n = 210$ ) compared to OB ( $14.8 \pm 3.85$ ,  $n = 194$ ), with distances being intermediate at NB ( $21.9 \pm 8.08$ ,  $n = 356$ ).

Residency varied with location in only 3/10 years (NB highest in 2/3 years, OB highest in 1/3 years), and overall residency was highest in OB ( $1.38 \pm 0.7$ ,  $n = 106$ ), intermediate in NB ( $1.31 \pm 0.57$ ,  $n = 168$ ), and lowest in MF ( $1.07 \pm 0.3$ ,  $n = 109$ ).

Similarly, female familiarity varied by location in 5/10 years (OB highest in 4/5 years, NB highest in 1/5 years) with mean female familiarity highest in OB ( $0.25 \pm 0.19$ ,  $n = 86$ ), intermediate in NB ( $0.21 \pm 0.2$ ,  $n = 102$ ), and lowest in MF ( $0.13 \pm 0.15$ ,  $n = 58$ ).

Network familiarity varied by location in 7/10 years (OB highest in 5/7 years, NB highest in 2/7 yrs), and overall network familiarity was highest in OB ( $0.34 \pm 0.17$ ,  $n = 127$ ), intermediate in NB ( $0.23 \pm 0.17$ ,  $n = 227$ ), and lowest in MF ( $0.19 \pm 0.14$ ,  $n = 123$ ).

Synchrony also varied by location in 11/11 years (OB greatest in 6/11 years, MF greatest in 4/11 years, NB greatest in 1/11 years;  $P < 0.051$  in all cases; Figure 2.5), with



NB exhibiting greater variance of laying (CV%,  $1.7 \pm 0.79$ ,  $n = 356$ ) than MF ( $1.54 \pm 0.73$ ,  $n = 210$ ) and OB ( $1.54 \pm 0.78$ ,  $n = 194$ ).

There was significant covariation among annual mean values for some social network metrics, with nearest neighbour distances being negatively correlated with residency and female -and- network familiarity metrics ( $P < 0.01$  in all cases). In contrast, residency and female -and- network familiarity were all positively correlated with each other ( $P < 0.001$  in all cases). However, there was no relationship between synchrony and any other social metric ( $P > 0.05$  in all cases, Table 2.3, 2.4).

In summary, MF (the linear nesting location) generally had a looser network structure with higher nearest neighbour distances, lower residency scores, and lower female and network familiarity compared to the two clumped nesting areas (OB and NB). However, despite this variation in social network structure among nesting locations, annual mean -and- temperature-predicted residual laying date were not related to annual means for any social network metric ( $P > 0.05$  in all cases, Table 2.3, 2.4, Figures 2.6-2.8); though there was a trend between synchrony and absolute laying date, with the degree of synchrony being lower with earlier laying ( $F_{1,31} = 3.72$ ,  $P = 0.06$ ; Figure 2.8).

#### Individual variation in laying date and social network metrics

As with co-variation of annual means (above), there was covariation in social network metrics among individuals: nearest nest distances were negatively correlated with residency and female -and- network familiarity. Residency and female -and- network familiarity were all positively correlated, however, synchrony was not related to any other social metric (Table 2.5). Although there was weak location variation in social network metrics, laying date was not associated with any measure of social network structure ( $P > 0.05$  in all cases, Table 2.6, Fig 2.9a-e).

Temperature-predicted residual laying date was associated with the interaction between nearest neighbour distances and nesting location ( $F_{2,754} = 3.16$ ,  $P = 0.04$ ), therefore we analysed this for each location separately. Individual variation in temperature-predicted laying date was dependent on nearest neighbour distance for birds nesting in MF, the linear habitat ( $F_{1,208} = 8.0$ ,  $P = 0.01$ ), with birds laying later than predicted by temperature with increasing neighbour distances. However, individual variation in temperature-predicted laying date was independent of nearest neighbour

distance in the two clumped habitats, NB ( $F_{1,354} = 0.1$ ,  $P = 0.75$ ) and OB ( $F_{1,192} = 0$ ,  $P = 1$ ; Figure 2.10a). There was no trait\*location interaction for any other network metrics ( $P > 0.05$  in all cases) therefore they were not subset into location for analysis. Neither residency or female familiarity were associated with temperature-predicted residual laying ( $P > 0.05$  in both cases; Figures 2.10a-e). Network familiarity was associated with temperature-predicted residual laying date at all locations ( $F_{6,470} = 2.55$ ,  $P = 0.02$ , Figure 2.10d), with networks comprised of 3/8 returning females laying closest to date predicted by temperature, particularly in comparison to 0 ( $P = 0.01$ ), 1 ( $P = 0.02$ ) and 2 ( $P = 0.01$ ) of 8 returning females. Females whose neighbours exhibited low degrees of synchrony laid earlier than predicted by temperature ( $F_{1,754} = 17.24$ ,  $P < 0.001$ , Figure 2.10e).

## Discussion

Here, we examined how absolute laying date and residual variation from temperature-predicted laying date were associated with overall spatial distribution of nest-boxes (i.e., “linear” vs “clumped”) and five measures of social network structure (neighbour distances, residency, female -and- network familiarity, and synchrony) at both a population- and individual-level in semi-colonial nesting European starlings. We found evidence of strong overall spatial structure with birds nesting in a linear habitat generally showing a ‘looser’ network structure (higher neighbour distances, lower residency and female -and- network familiarity) than females nesting in clumped habitats. In addition, there was covariation between four of the social network measures, with the exception of synchrony. However, variation in social network structure among nesting locations with different overall spatial structure was not associated with annual or individual variation in laying date nor with annual deviation from the temperature-predicted laying date. Individual variation in residual, temperature-independent laying date was associated with a) nearest neighbour distances in the linear habitat, with females at greater distances from neighbours laying later than predicted by temperature, and b) network familiarity, with networks with more returning females (3/8) laying closest to the predicted date, but was independent of residency or female familiarity. Finally, despite the fact that synchrony was not associated with other social network metrics, females in networks with low synchrony among neighbours laid earlier than predicted by temperature.

We found evidence of strong differences in social structure in all five network measures among different nesting locations. Females nesting in a linear habitat (Main Field) had greater nearest neighbour distances but lower residency and female and network familiarity than those in clumped habitats (Old Barn and New Barn). Although synchrony also varied by nesting location this was not consistent with overall spatial distribution: females nesting in a clumped (Old Barn) and linear habitat (Main Field) exhibited marginally more synchronous laying compared to a second, clumped, nesting location (New Barn). Similarly, there was significant covariation among our four social network measures: females with greater nearest neighbour distances had lower residency scores and lower female -and- network familiarity. However, each of these measures were unrelated to our measure of synchrony. While it seems intuitive that nearest neighbour distances, residency and familiarity of females in a nesting area, established during the pre-breeding period (Henry et al., 2013), could *predict* subsequent patterns of egg-laying, synchrony is more likely an emergent property of the female's laying decisions. It is also possible that highly synchronized females are more likely to remain together in subsequent years, although Riehl and Strong (2018) found no evidence for this in greater anis (*Crotophaga major*).

The majority of studies to date have focused on synchrony of breeding (not timing, or initiation, of laying), and mainly in relation to fledging or breeding success. For example, in guillemots (*Uria aalga*) breeding synchrony was related to nesting density, categorized as overall spatial distribution of nesting (dense, medium, sparse) on different sized cliff ledges, and breeding synchrony was positively correlated with breeding success (Birkhead, 1977). Similarly, in European starlings (Evans et al., 2009) and carrion crows, *Corvus corone* (Yom-Tov, 1975), females nesting at higher density or with lower inter-nest distances had more synchronous laying and higher fledging or breeding success. In these studies, "clumped" versus "dispersed" nesting likely reflected higher nest density and lower nearest nest distances, respectively, even though these were not directly measured, but they rarely consider residency or familiarity of nesting females. However, in Cory's shearwaters, *Calonectris diomedea*, Mougín et al. (2001) showed that females that nested closer to neighbours (1-2 m) were subsequently more synchronized than birds that nested further apart (~6 m). Furthermore, Westneat (1992) showed that the temporal proximity of other nests (number of other nests started within  $\pm$  2 days) predicted fledging success in red-winged blackbirds (*Agelaius phoeniceus*).

Finally, in cooperatively-breeding anis Riehl and Strong (2018) showed that females that had previously nested together synchronized their communal egg-laying more rapidly which decreased competition and resulted in higher overall fledging success. In contrast, we found that overall spatial distribution and nearest neighbour distances did not influence the level of synchrony in a network which suggests that synchrony arises as a result of other factors (i.e., females breeding at optimal times, a threshold lifted allowing birds to begin laying, low degrees of stress in the network (Helm et al., 2006; Jovani and Grimm, 2008; Riehl and Strong, 2018; Westneat, 1992)), and, although not selected to breed synchronously *per se*, females can benefit from initiating laying at the same time (e.g. through group-foraging, predator defense, greater information pooling). Therefore, when investigating the role of social factors as supplemental cues, synchrony may not be an appropriate measure to use when examining the relationship between social networks and timing of laying.

Fewer studies have considered synchrony, or other social networks metrics, in relation to timing of initiation of egg-laying (i.e. our main focus). Some studies have reported relationships between earlier laying and social factors such as increased social stimulation through vocalizations (Waas et al., 2005) or prior experience on the breeding ground (Pitera et al., 2001; Westneat, 1992). However, even where synchrony predicts breeding success most studies have failed to find significant effects of synchrony or nearest nest distances on laying date (Birkhead, 1977; Mougín et al., 2001; Yom-Tov, 1975). Our results for absolute laying date are consistent with this: synchrony did not predict annual or individual variation in laying date. Whole-colony social interactions may result in decreased reliability of “public information” (i.e., decreased social stimulation and information degradation, microvariation in habitat, resource availability, social structures within sub-colonies, year-to-year variation) which may explain why social factors were not associated with annual laying date. In contrast, in another study of European starlings Evans et al. (2009) showed that females nesting at higher density not only had higher synchrony but also initiated egg-laying earlier. However, in our study females in networks with lower synchrony among neighbours laid earlier than predicted by temperature, i.e. synchrony explained residual temperature-independent laying date, which may be a by-product of natural spreads in laying, as the ‘peak’ of laying in European starlings typically occurs only 3-4 days after the first egg is laid. In the only study we are aware of that considered female familiarity in social networks, Gabrowski-

Zhang et al. (2011) determined the number of neighbours that female great tits (*Parus major*) had shared territory boundaries with the year before. Females had from 0 to 11 neighbors with whom they were familiar and females with more familiar individuals in their neighborhood laid larger clutches but there was no effect on laying date. Westneat (1992) also found that females with greater familiarity of their breeding environment – similar to our residency – laid earlier than unfamiliar females resulting in less synchronous laying of prior residents. In agreement with the majority of these studies, we found no effect of social network metrics on variation in absolute laying date, either at the individual or annual level.

However, in our study individual variation in residual temperature-independent laying date was associated with network familiarity, with networks comprised of 3/8 returning females laying closest to that predicted by temperature, regardless of these female's 'familiarity'. Only in the linear habitat did increasing neighbour distances lead to individuals laying later than predicted by temperature, which may be associated with correlations among social metrics, as females at greater distances show decreased network familiarity potentially resulting in limited interactions with other females (Immer et al., 2021; Westneat, 1992), decreased visual and auditory stimulation (Ball and Ketterson, 2008; Helm et al., 2006), and dissipation of the network. In linear habitats with larger neighbour distances, it may be more beneficial to rely on prior personal experience (Immer et al., 2021) or initiate laying later in response to reduced information gathering at a larger scale. As females in networks with 3/8 returning females laid closest to temperature-predicted laying date, it is possible that a network incorporating both new and returning females may allow for a greater diversity of information from conspecifics, with returning females providing information based on prior experience (i.e., habitat characteristics, resource availability and locations, predators) and new recruits providing novel information from their surroundings or potentially affecting the network dynamics (i.e., competition, territoriality, stress levels). Another possibility is that juveniles return with and gather information from more experienced females and follow their cues (Doligez et al., 2003).

In summary, our study suggests that some components of social network structure can explain residual, temperature-independent variation in laying date and could therefore act as a supplemental cue for timing of egg-laying. As no studies have investigated the relationship between temperature-predicted residual laying date and

social factors, or other supplemental cues in general, there is no study to compare these results to, so it is important to interpret this with caution. We posit that the correlations between network familiarity and neighbour distances, residency, and female familiarity may explain inconsistencies among studies examining social factors. Additionally, only when we considered temperature-predicted residual laying date did we find any relationship between social networks and timing of egg-laying which may explain why previous studies failed to find associations between social factors and absolute laying date (Birkhead, 1977; Mougin et al., 2001; Yom-Tov, 1975). These results exemplify the need to include temperature-predicted residual laying date and network familiarity in analysis to yield more accurate information about the mechanisms females use to fine-tune laying date to the local environment. An intriguing further step would be to examine if network structure and temperature-predicted residual laying date interact to influence reproductive success.

## General conclusion

In this thesis, I firstly found that temperature provides a relatively long-term cue for timing of egg-laying in European starlings (*Sturnus vulgaris*), with temperatures from mid-winter (January/February) to the immediate pre-breeding period (March) being most informative of variation in laying date. This is an important, novel result as it contradicts the widely held idea that short-term temperatures, just prior to egg-laying act as “supplemental cues” fine-tuning the female’s laying decision to local environmental conditions (e.g. Brommer et al., 2008; Pearce-Higgins et al., 2005; Schaper et al., 2012). Secondly, I found some evidence that certain measures of female social networks explained variation in laying date but only when I considered temperature-predicted residual laying date. A lack of relationship between social network cues and absolute timing of egg-laying may explain why previous studies failed to find associations between social factors and absolute laying date (e.g. Birkhead, 1977; Mougin et al., 2001; Yom-Tov, 1975). I found that residual, temperature-independent laying date was associated with overall network familiarity and nearest neighbour distances in a “linear” habitat which suggests that some component of female-female social network structure could act as a supplemental cue for timing of egg-laying. To our knowledge, ours is the first study investigating how social factors might contribute to deviation from the temperature-predicted laying date.

Investigating how supplemental cues are associated with different aspects of phenology has been a long-standing topic of interest, however our knowledge of supplemental cues, including temperature I would argue, is patchy, as the temperature window most relevant to reproductive timing remains unknown (Wiebe and Gerstmar, 2010). Many studies examining the relationship between temperatures and laying date have restricted their analysis to temperatures associated with the breeding seasons of the study species (e.g., Jeong et al., 2020; Shave et al., 2019; Smith et al., 2020; Watts et al., 2019; Wesolowski et al., 2021) but this is often done with little *a priori* knowledge on the relationship between climate and laying date, leading to arbitrary selections of climate windows (Bailey and van de Pol, 2016; Pol et al., 2016). As indicated here, it is possible that temperatures do not act as supplementary cues fine-tuning timing of egg-laying rather they are more similar to photoperiod in either providing a broad window in which to time laying, or being integrated over a long time-period. It would be useful for

previous analyses that limited their window of selection to re-run initial analyses using all temperature windows to examine if this changes their results. In addition, studies should expand their temperature analyses to include residual, temperature-independent laying date so they are not only examining deviation from average conditions, but rather examine how organisms are *responding* to temperature changes and how supplemental cues contribute to deviation from the temperature-predicted laying date. As I found that mixed networks with 'new' and 'returning' females laid closest to the temperature-predicted date, it would be interesting to explore if network structure and temperature-predicted residual laying date interact to influence reproductive success. My thesis research suggests two key questions: a) what component of information on long-term temperature cues do birds utilise to time their laying decisions (e.g. does this involve direct effects of temperature or indirect effects via prey or vegetation phenology; Chmura et al., 2020), and b) what component of female-female social information do birds utilise to time their laying decision (e.g. is this song, or visual cues during social interactions)?

For the first question, prey availability and abundance has also been considered a supplemental cue as temperature-dependent (ectothermic) organisms such as insects are directly affected by temperature, perhaps providing an indirect link between temperature and reproductive parameters (Pearce-Higgins et al., 2010). Food availability and quality signals the amount of nutrients, proteins, and energy available for the physiological task of preparing for reproduction, state of nutrients that will be available when provisioning young, and subsequent energy for molting which is considered vital for avian fitness. Therefore, food supply must be perceived as ample before, during, and after breeding in order to provide the energy required for reproductive success (Schaper et al., 2012). At Davistead Farm, starlings' main food source provided to their young are soil larvae, mostly consisting of tipulid (cranefly) larvae (Williams et al., 2015). The growth rate, size of larvae, and timing of development are suggested to be determined by temperature exposure in their early stage of development (Pritchard, 1983). Peak tipulid larval biomass extends for approximately a 30-40-day window, which appears to be when the starlings at Davistead aim to be provisioning young (Williams et al., 2015). Williams et al. (2015) found that mid-winter temperatures (8 Jan – 22 Feb) best predicted laying date in this population of starlings and suggested that they use their bill to probe the soil and assess the stage of development of larva during winter conditions. Future research in this same population could incorporate data collected on tipulid size



and abundance at the study site to examine how prey availability may act as a supplemental cue. This could be analysed by examining how temperature predicts tipulid size using temperature windows discussed in Chapter 1 or running a sliding window analysis with temperature and first brood prey size to examine if this relationship could act as a predictive cue by signalling the state of nutrient availability during provisioning. Additionally, one could examine if there is a trend between tipulid size during midwinter, pre-breeding, and first broods that may act as a predictive cue on availability or quality of prey.

Turning to the second question of what component of female-female social information birds utilise to time their laying decision, most studies to date have focused on males, and there is much to be explored about female social networks as supplemental cues (Williams, 2012). There is only limited information on social metrics (cf. synchrony) and this could be examined in a wide range of taxa with different social dynamics (i.e., solitary, eusocial, seasonally social, (semi) colonial). Importantly, given the strong influence of temperature there appear to have been no studies examining the relationship between social structure and temperature-independent laying (i.e. residual variation). Cornell et al. (2017) conducted a playback experiment using male vocalisations at Davistead Farm to examine if this effected laying date. While they did find that male song increased pre-breeding activity there was no effect on laying date. Research has shown that female vocalisations stimulate ovarian development and reproductive behaviour (Cheng et al., 1998; Waas et al., 2005). It would be interesting to conduct a playback experiment at Davistead Farm using female vocalisations to examine if this could influence the laying date of European starlings by inducing positive social stimulation (advancing laying) or negative social stimulation (delaying laying date). It is apparent that much can be done to advance our knowledge on the supplemental cues that fine-tune laying ranging from a simple re-analysis of temperature data to include all temperature windows, calculate and analyse temperature-predicted residual laying date, more thoroughly examine prey as a supplemental cue, run more exhaustive female social network analysis that includes network familiarity, perform field experiments, and extend analysis to a broad range of taxa.

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## Tables

**Table 2.1 Relationships between all nearest neighbor distances 1-7 (dist\_n), mean distances of neighbors 1-3 (x3dist\_indiv), and mean distances of neighbors 1-7 (x7dist\_indiv). Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Sample size indicated by number in parentheses.**

	dist_1	dist_2	dist_3	dist_4	dist_5	dist_6	dist_7	x3dist_indiv	x7dist_indiv
dist_1	1	<b>0.82***</b> (760)	<b>0.71***</b> (760)	<b>0.61***</b> (760)	<b>0.58***</b> (760)	<b>0.55***</b> (760)	<b>0.53***</b> (760)	<b>0.87***</b> (760)	<b>0.68***</b> (760)
dist_2		1	<b>0.89***</b> (760)	<b>0.81***</b> (760)	<b>0.78***</b> (760)	<b>0.73***</b> (760)	<b>0.72***</b> (760)	<b>0.97***</b> (760)	<b>0.85***</b> (760)
dist_3			1	<b>0.94***</b> (760)	<b>0.91***</b> (760)	<b>0.86***</b> (760)	<b>0.85***</b> (760)	<b>0.95***</b> (760)	<b>0.95***</b> (760)
dist_4				1	<b>0.98***</b> (760)	<b>0.93***</b> (760)	<b>0.92***</b> (760)	<b>0.87***</b> (760)	<b>0.98***</b> (760)
dist_5					1	<b>0.96***</b> (760)	<b>0.95***</b> (760)	<b>0.84***</b> (760)	<b>0.98***</b> (760)
dist_6						1	<b>0.99***</b> (760)	<b>0.79***</b> (760)	<b>0.97***</b> (760)
dist_7							1	<b>0.78***</b> (760)	<b>0.96***</b> (760)
x3dist_indiv								1	<b>0.91***</b> (760)
x7dist_indiv									1

**Table 2.2 Annual and location variation in absolute laying date (LD), temperature-independent laying date (Ta-LD), nearest neighbour distances 1-7, residency 1-3, female -and- network familiarity, and synchrony (2011-2021). n = sample size.**

Trait	Year (F, P, n)	Loc (F, P, n)	Year*Loc (F, P, n)
LD	$F_{10} = 415.48, < 0.001, 760$	$F_2 = 3.82, 0.02, 760$	$F_{20} = 2.16, 0.003, 760$
Ta-LD	$F_{10} = 103.43, < 0.001, 760$	$F_2 = 3.82, 0.02, 760$	$F_{20} = 2.16, 0.003, 760$
NN7 (not logged)	$F_{10} = 7.84, < 0.001, 760$	$F_2 = 75.61, < 0.001, 760$	$F_{20} = 5.53, < 0.001, 760$
Res 1-3	$F_9 = 1.46, 0.16, 383$	$F_2 = 5.26, 0.01, 383$	$F_{18} = 1.69, 0.04, 383$
Female fam	$F_9 = 2.01, 0.04, 246$	$F_2 = 5.64, 0.01, 246$	$F_{18} = 3.59, < 0.001, 246$
Network fam	$F_9 = 6.01, < 0.001, 477$	$F_2 = 13.4, < 0.001, 477$	$F_{18} = 8.48, < 0.001, 477$
Synchrony	$F_{10} = 20.9, < 0.001, 760$	$F_2 = 6.1, 0.002, 760$	$F_{20} = 8.35, < 0.001, 760$

**Table 2.3 Relationships between mean laying date (xdate), temperature-predicted residual laying date (Ta\_devxLD), mean neighbour distances 1-7, mean residency 1-3, mean female -and- network familiarity, and synchrony (2011-2021). Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Sample size indicated by number in parentheses.**

	xdate	Ta_devxLD	xNN1-7	xRes1_3	xFemale fam	xNetwork fam	Synchrony
xdate	1	<b>0.51* (20)</b>	-0.25 (11)	0.28 (10)	0.33 (10)	0.03 (10)	-0.56 (11)
Ta_devxLD		1	0.11 (11)	0.38 (10)	0.59 (10)	0.3 (10)	-0.38 (11)
Mean_NND1_7			1	0.27 (10)	-0.17 (10)	-0.24 (10)	-0.13 (11)
xRes1_3				1	0.57 (10)	0.33 (10)	<b>-0.66* (10)</b>
Female familiarity					1	<b>0.64* (10)</b>	-0.34 (10)
Network familiarity						1	0.16 (10)
Synchrony							1

**Table 2.4 Relationships between mean laying date (LocxLD), temperature-predicted residual laying date (LocTa\_devLD), mean neighbour distances 1-7 (LocxNN1\_7), mean residency 1-3, mean female -and- network familiarity, and synchrony by year\*loc (2011-2021). Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Sample size indicated by number in parentheses.**

	LocxLD	LocTa_devLD	xNN1-7	xResidency 1-3	xFemale fam	xNetwork fam	xSynch
LocxLD	1	<b>0.64***</b> (33)	-0.06 (33)	0.17 (30)	0.12 (30)	0.07 (30)	-0.33 (33)
LocTa_devLD		1	-0.01 (33)	0.2 (30)	0.25 (30)	0.18 (30)	-0.18 (33)
LocxNN1_7			1	<b>-0.64***</b> (30)	<b>-0.44**</b> (30)	<b>-0.45**</b> (30)	-0.05 (33)
xRes1_3				1	<b>0.65***</b> (30)	<b>0.65***</b> (30)	-0.26 (30)
Female familiarity					1	<b>0.79***</b> (30)	-0.22 (30)
Network familiarity						1	-0.08 (30)
Synchrony							1



**Table 2.5 Relationships between individual residual laying date (residLD), temperature-predicted residual laying date (Ta\_residLD), neighbour distances 1-7 (x7dist\_indiv), residency 1-3, female -and- network familiarity, and synchrony (2011-2021). Values are Pearson's correlation coefficients and asterisks indicate significance level (\* < 0.05, \*\* < 0.01, \*\*\* < 0.001). Sample size indicated by number in parentheses.**

	residLD	Ta_residLD	x7dist_indiv	xResidency1_3	xFemale fam	xNetwork fam	xSynch
residLD	1	<b>0.67***</b> (760)	-0.02 (760)	-0.03 (383)	-0.06 (246)	0.08 (477)	-0.03 (760)
Ta_residLD		1	0 (760)	0.03 (383)	0.09 (246)	<b>0.12**</b> (477)	<b>-0.15***</b> (760)
x7dist_indiv			1	<b>-0.19***</b> (383)	<b>-0.24***</b> (246)	<b>-0.21***</b> (477)	-0.03 (760)
Residency				1	<b>0.35***</b> (186)	<b>0.35***</b> (377)	-0.03 (383)
Female familiarity					1	<b>0.81***</b> (199)	-0.04 (246)
Network familiarity						1	0.01 (477)
Synchrony							1

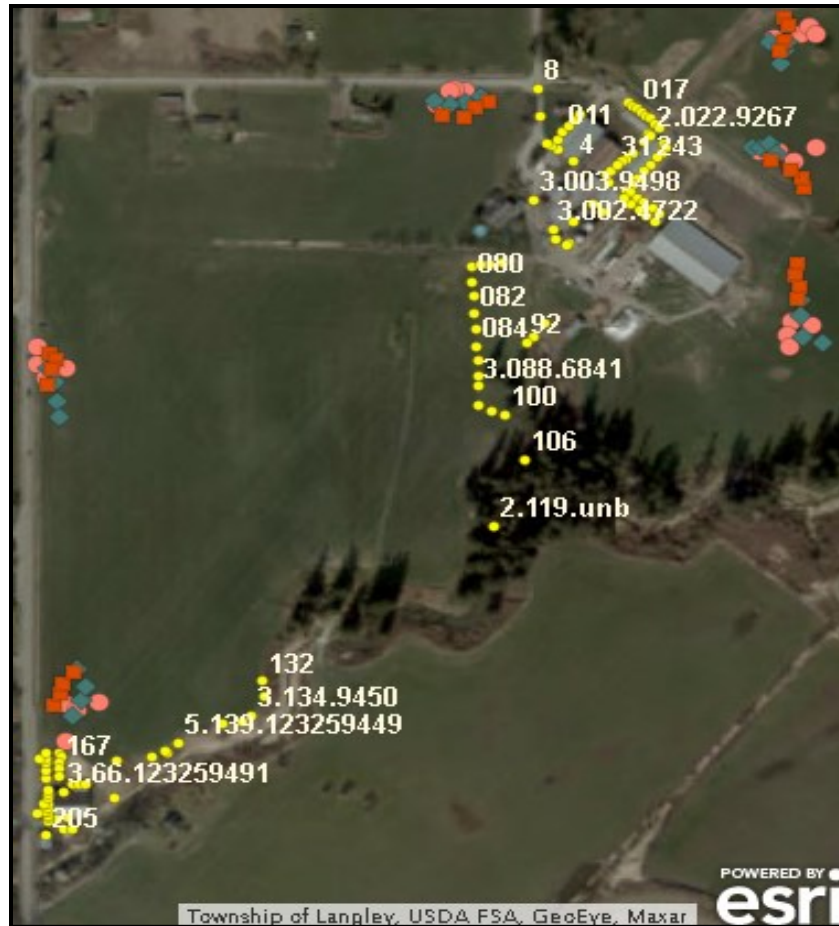
**Table 2.6 Association of individual residual laying date with nearest neighbour distances 1-7, residency 1-3, female -and- network familiarity, and synchrony by location (2011-2021). n = sample size.**

	Trait (F, P, n)	Loc (F, P, n)	Trait*Loc (F, P, n)
NN 1-7	F <sub>1</sub> = 0.67, 0.41, 766	F <sub>2</sub> = 3.06, 0.05, 766	F <sub>2</sub> = 2.04, 0.13, 766
Residency	F <sub>2</sub> = 1.35, 0.26, 383	F <sub>2</sub> = 1.03, 0.36, 383	F <sub>4</sub> = 1.42, 0.23, 383
Female familiarity	F <sub>5</sub> = 1.32, 0.26, 246	F <sub>2</sub> = 3.7, 0.03, 246	F <sub>9</sub> = 1.12, 0.35, 246
Network familiarity	F <sub>6</sub> 1.04, 0.4, 483	F <sub>2</sub> = 1.15, 0.32, 483	F <sub>11</sub> = 0.54, 0.88, 483
Synchrony (cvLD)	F <sub>1</sub> = 0.04, 0.85, 766	F <sub>2</sub> = 2.12, 0.12, 766	F <sub>2</sub> = 0.28, 0.76, 766

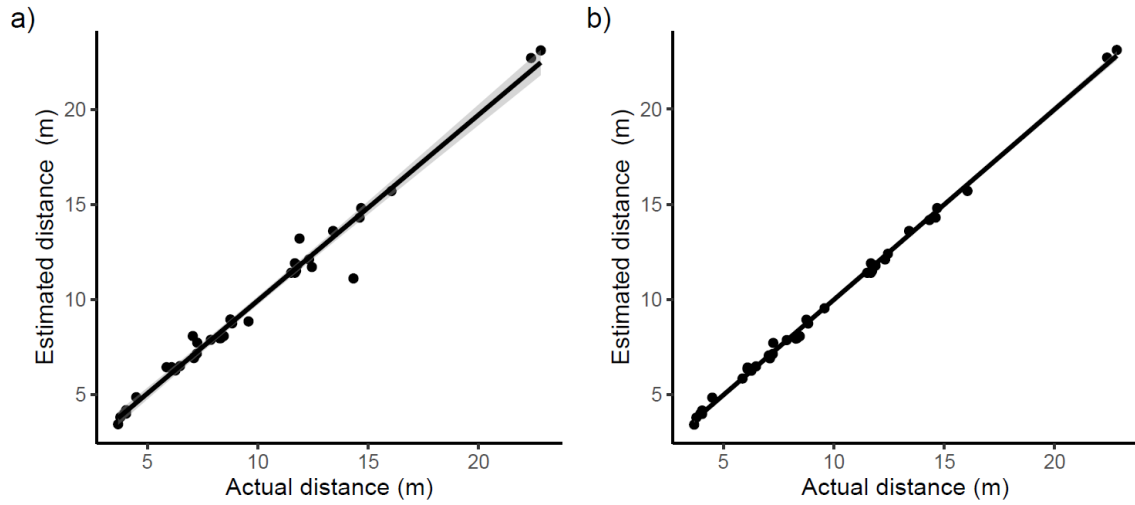
**Table 2.7 Association of individual temperature-predicted residual laying date with nearest neighbour distances 1-7, residency 1-3, female -and- network familiarity, and synchrony by location (2011-2021). n = sample size.**

	Trait (F, P, n)	Loc (F, P, n)	Trait*Loc (F, P, n)
NN 1-7	$F_{1,754} = 0.47, 0.49, 760$	$F_{2,754} = 3.84, 0.02, 760$	$F_{2,754} = 3.16, 0.04, 760$
Residency	$F_{2,374} = 0.21, 0.81, 383$	$F_{2,374} = 0.27, 0.76, 383$	$F_{4,374} = 0.82, 0.51, 383$
Female familiarity	$F_{5,229} = 1.85, 0.1, 246$	$F_{2,229} = 0.28, 0.76, 246$	$F_{9,229} = 0.76, 0.66, 246$
Network familiarity	$F_{6,457} = 2.54, 0.02, 477$	$F_{2,457} = 0.74, 0.48, 477$	$F_{11,457} = 0.79, 0.65, 477$
Synchrony (cvLD)	$F_{1,754} = 17.24, < 0.001, 760$	$F_{2,754} = 2.9, 0.05, 760$	$F_{2,754} = 1.4, 0.25, 760$

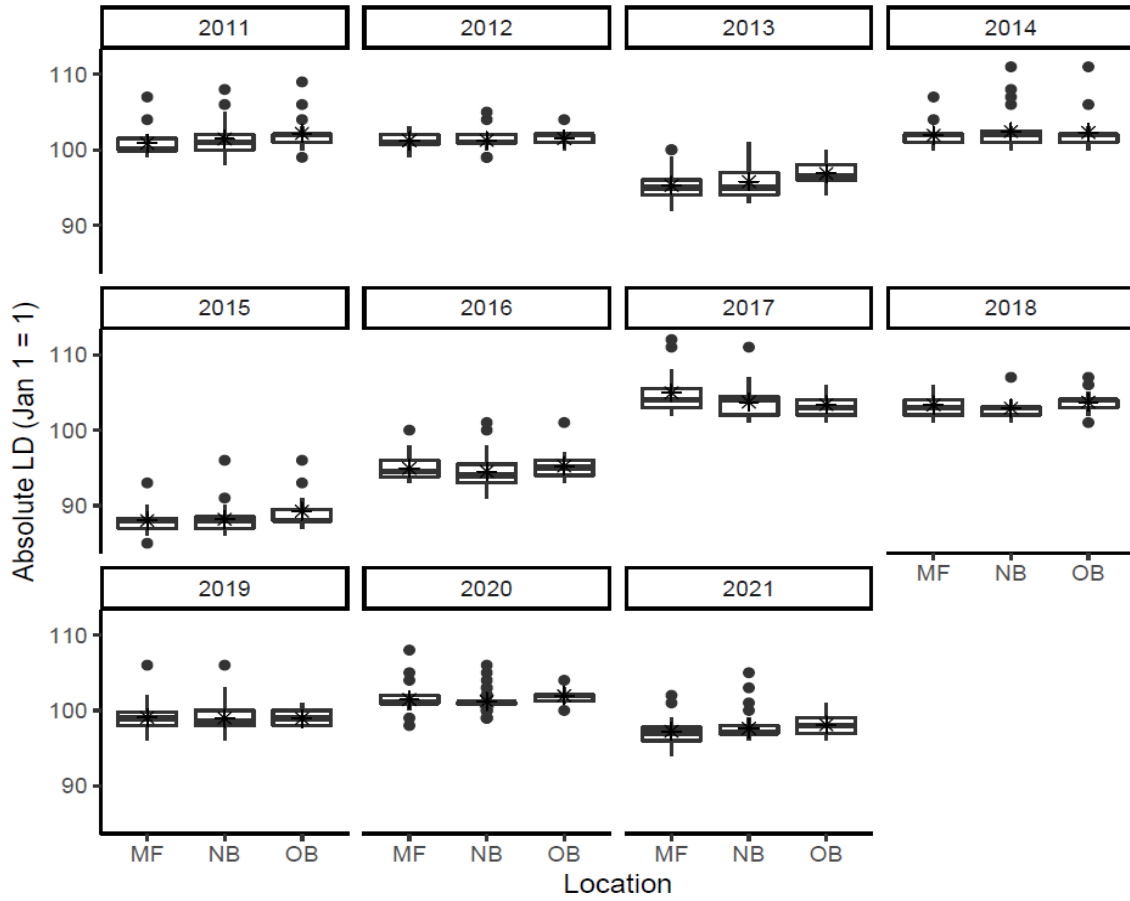
## Figures



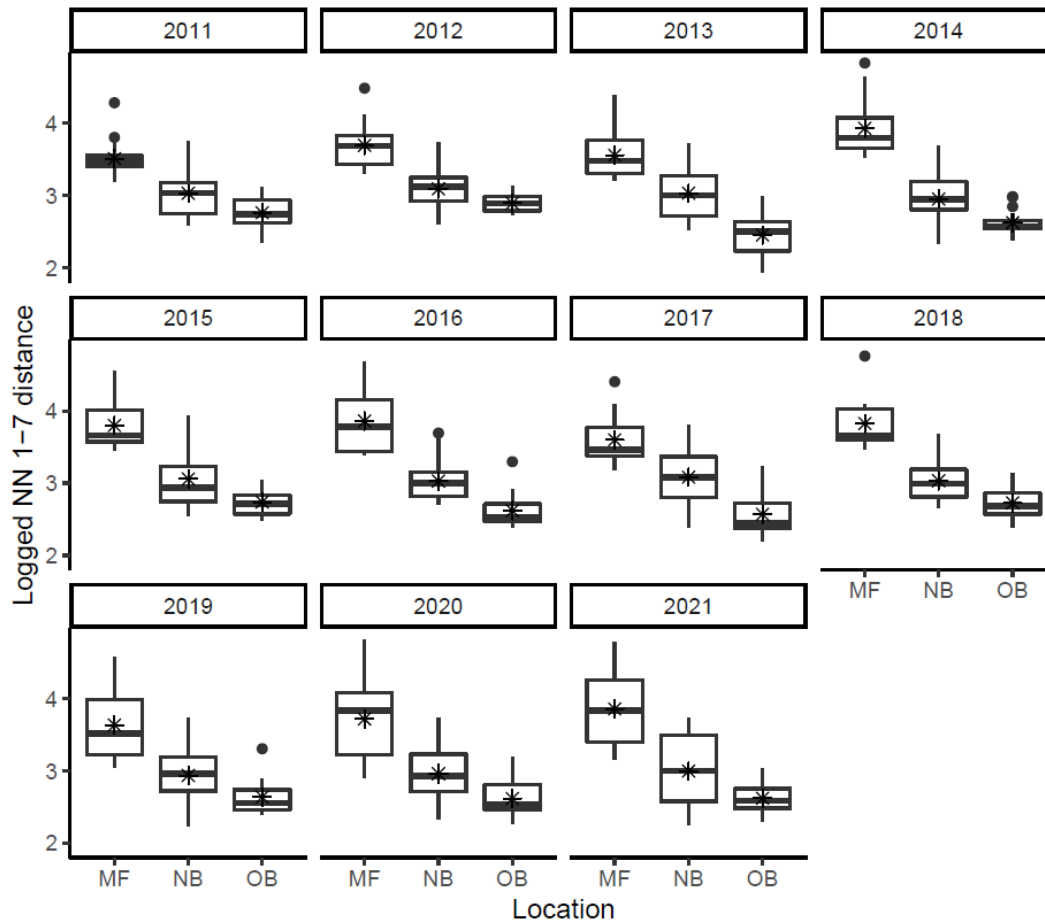
**Figure 2.1** Map of our study site, Davistead Farm in Langley, British Columbia, showing the two 'clumped' habitats at opposite ends of the farm (Old Barn lower left, New Barn upper right) and the 'linear' habitat (Main Field; middle). Yellow dots indicate nest-boxes and numbers to the right provide an example of unoccupied boxes (nest-box #), boxes that failed before onset of incubation (#, box#, unb), and banded females (#, box#, band#). Red, pink, and green shapes represent soil sampling locations which is not discussed here. Sources: Esri, Township of Langley, United States Department of Agriculture Farm Service Agency, GeoEye, Maxar. ArcGIS Online map hosted by ©Esri.



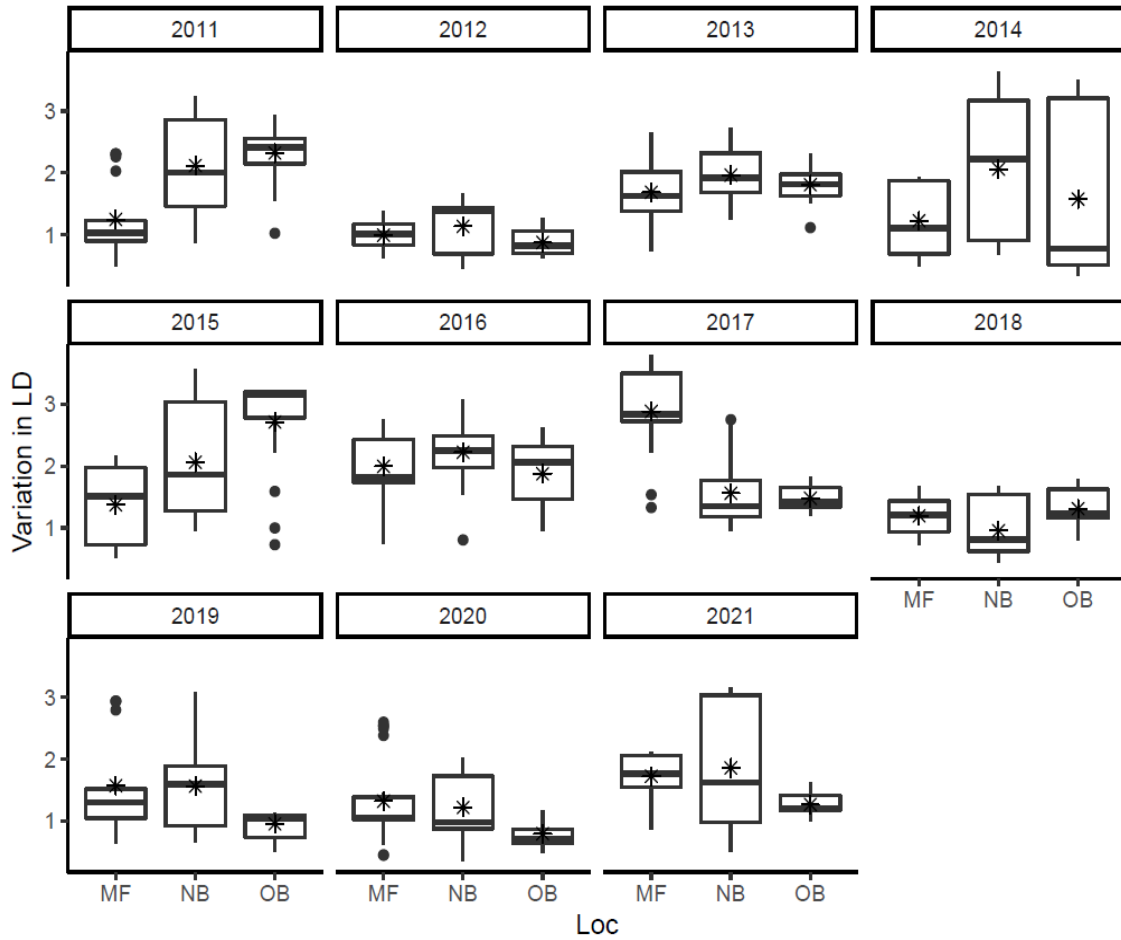
**Figure 2.2** Actual vs estimated ArcGIS nest-box distances a) before correcting outliers ( $r^2 = 0.98$ ,  $P < 0.001$ ) and b) after correcting outliers ( $r^2 = 1$ ,  $P < 0.001$ ) used to 'groundtruth' distances.



**Figure 2.3** Absolute laying date (clutch initiation date based on Julian calendar; 1 January = 1) of European starlings by year (2011-2021) and location ('linear' = MF, 'clumped' = NB, OB).

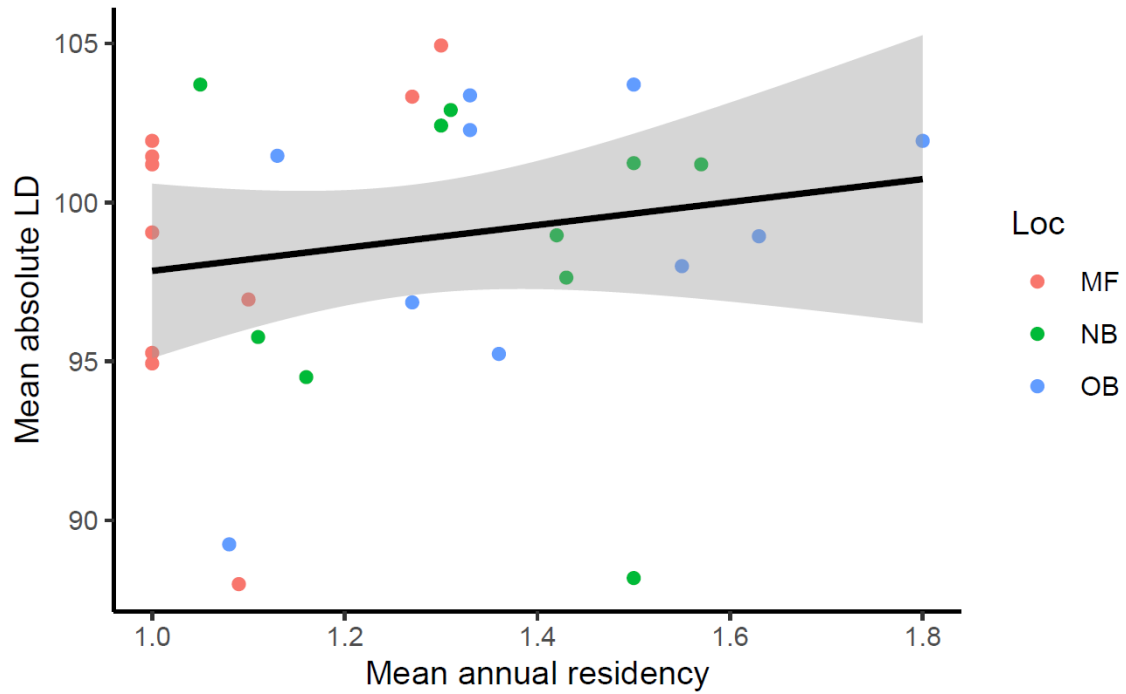


**Figure 2.4** Mean distances (logged) to nearest neighbours 1-7 by year (2011-2021) and location ('linear' = MF; 'clumped' = NB, OB) nearest neighbor distances 1-7 by year and location (2011-2021).

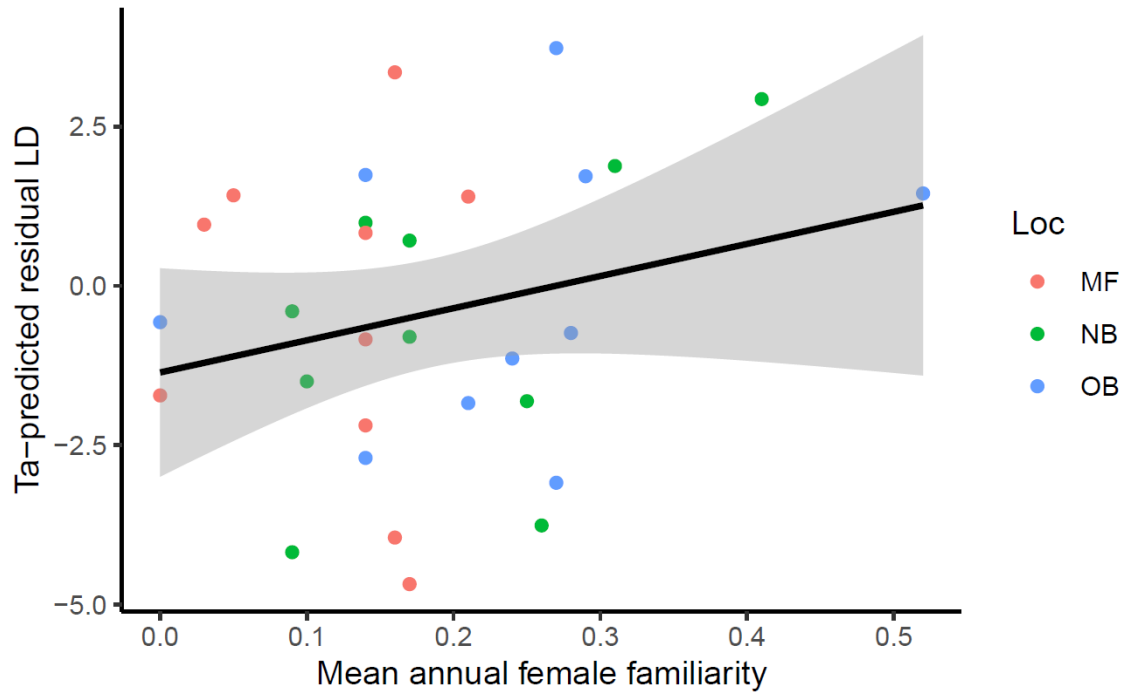


**Figure 2.5 Synchrony in laying (coefficient of variation) in starling networks by year (2011-2021) and location ('linear' = MF, 'clumped' = NB, OB). Higher y-axis values represent lower levels of synchrony.**

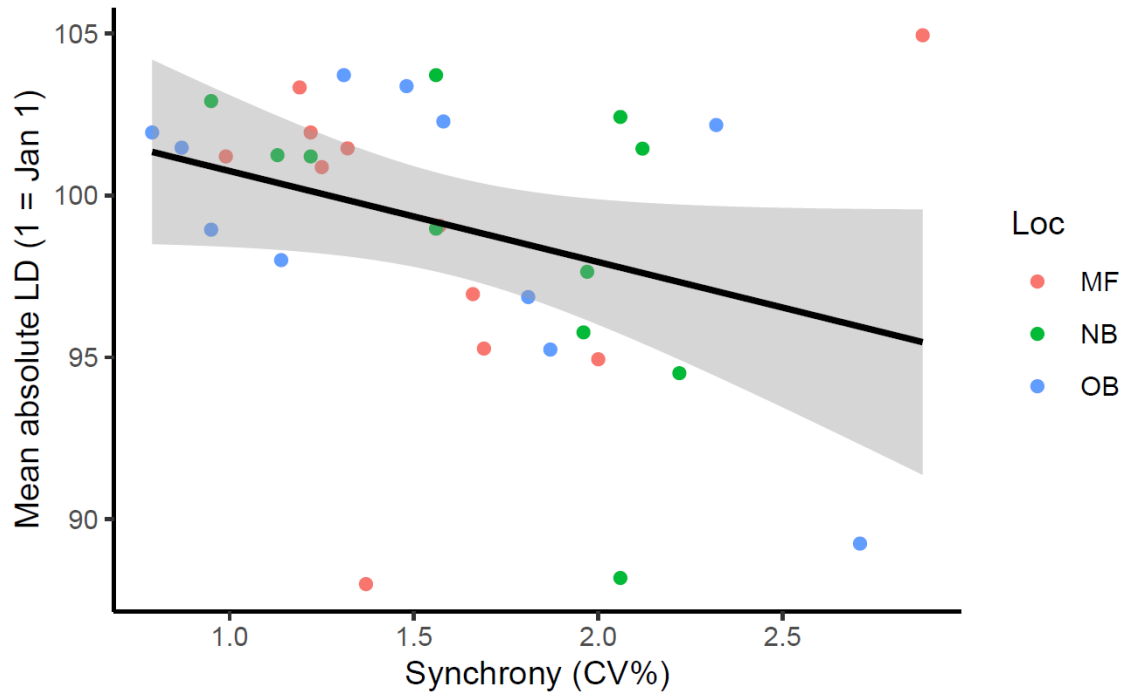




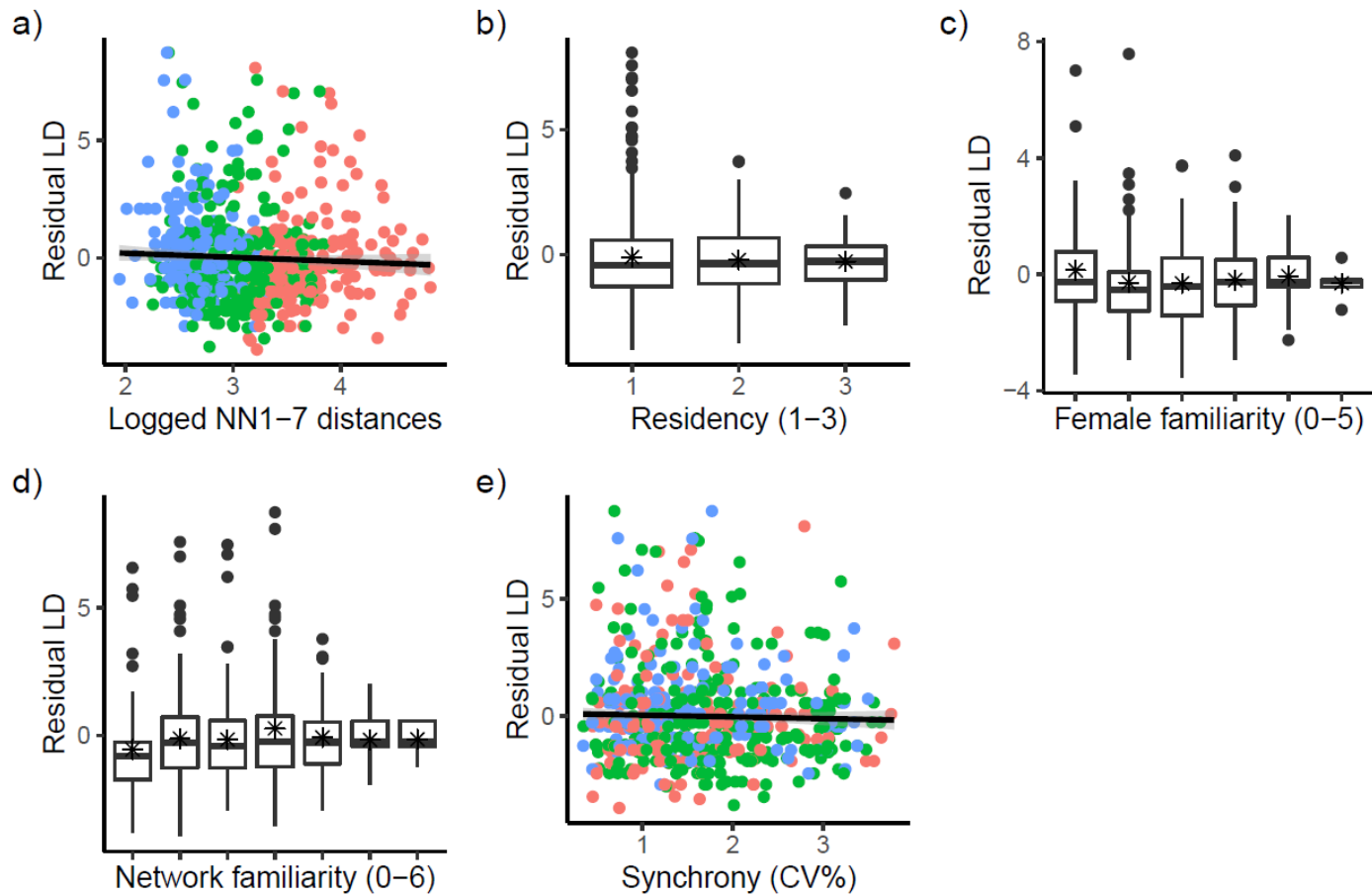
**Figure 2.6** The relationship between annual mean residency and annual mean absolute laying date in starlings by year and location (2012-2021,  $n = 30$ ,  $P = 0.33$ ). Colours indicate location (pink = MF, green = NB, blue = OB).



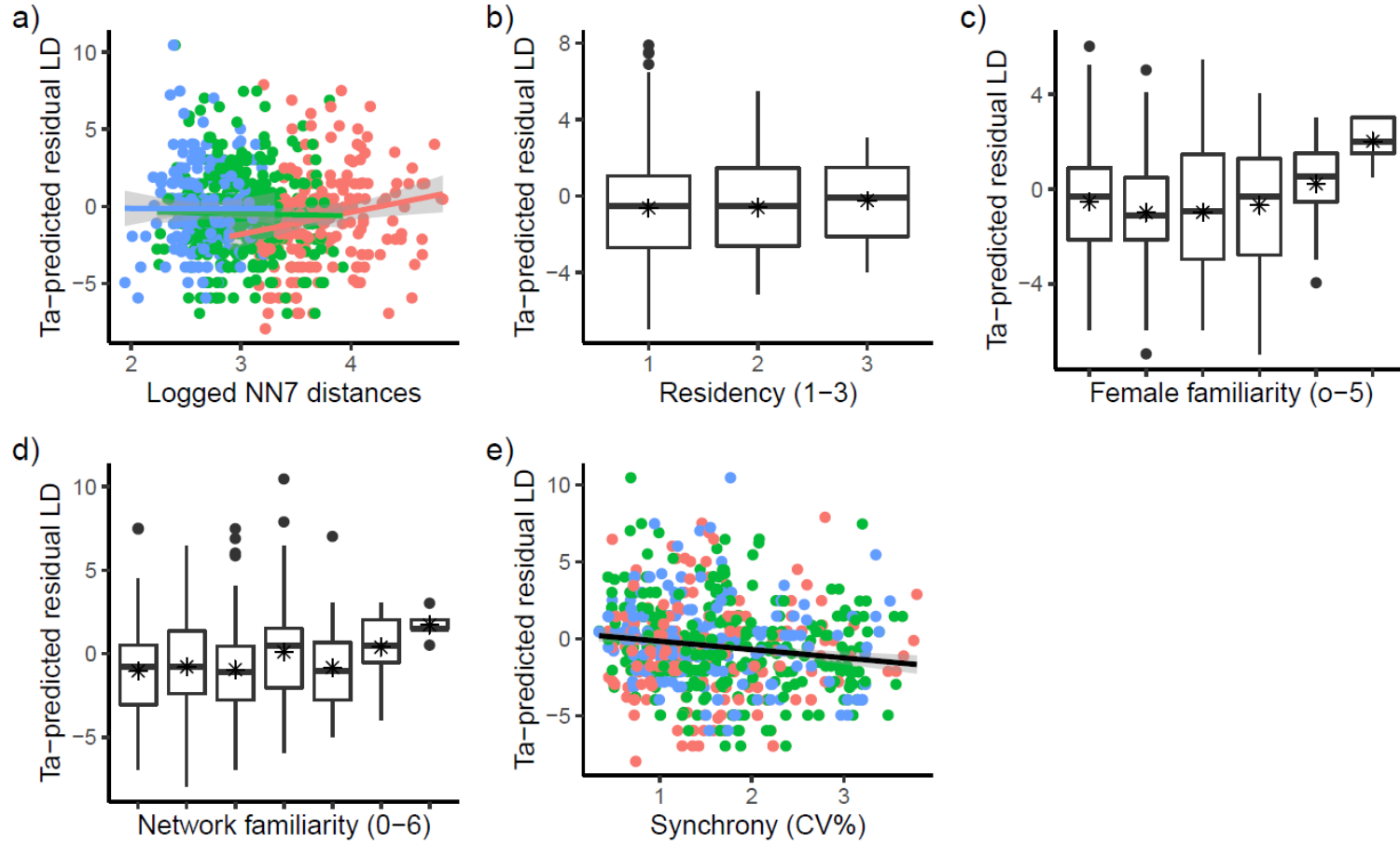
**Figure 2.7** The relationship between annual mean female familiarity and annual temperature-predicted residual laying date by year and location (2012-2021,  $n = 30$ ,  $P = 0.18$ ). Colours indicate nesting location (pink = MF, green = NB, blue = OB).



**Figure 2.8** The relationship between annual mean synchrony (lower values = higher synchrony) and mean absolute laying date at the level of year and location (2011-2021,  $n = 33$ ,  $P = 0.06$ ). Colours indicate nesting location (clumped: NB = green, OB = blue; linear: MF = pink).



**Figure 2.9** The relationship between individual residual laying date and a) logged nearest neighbour distances 1-7, b) residency 1-3, c) female familiarity 0-5, d) network familiarity 0-6, and e) synchrony in laying (2011-2021). Colours represent location (clumped: NB = green, OB = blue; linear: MF = pink).



**Figure 2.10** The relationship between individual temperature-predicted residual laying date and a) logged nearest neighbour distances 1-7, b) residency 1-3, c) female familiarity 0-5, d) network familiarity 0-6, e) synchrony in laying (2011-2021). Colours represent location (clumped: NB = green, OB = blue; linear: MF = pink).