PRENATAL REPRODUCTIVE SUCCESS OF AN AERIAL INSECTIVORE, THE TREE SWALLOW (*TACHYCINETA BICOLOR*)

by

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Abstract

Worldwide, many avian species are declining as the effects of large-scale, anthropogenic pressures continue to rise. Prenatal mortality is potentially an important driver of demographic processes in animal populations, yet little is known about embryonic mortality in birds, despite extensive research into factors driving mortality in the nestling, fledgling, and adult life stages. Hatching failure in avian species is common, and because many species are globally declining, assessing the processes that operate during all life stages of individuals, including prenatally, is important to fully understand demographic changes. My thesis investigated hatching failure and prenatal reproductive success in tree swallows (*Tachycineta bicolor*), a member of the aerial insectivorous guild which is rapidly declining across North America. Using fluorescence microscopy to identify embryonic and sperm nuclei, I first determined that although 10.1% of eggs failed to hatch (excluding abandoned, broken, and depredated eggs), 98.3% to 100% of eggs from a population of tree swallows breeding on three sites near Prince George, British Columbia, Canada, were fertile, and that the majority of hatching failure was due to embryonic mortality, not infertility. Embryonic mortality was most likely to occur during early incubation before development was visible macroscopically. I then assessed extrinsic (e.g., environmental conditions) and intrinsic (e.g., female age) factors that influence egg mass and embryonic viability (i.e., survival of an embryo to hatching). Egg mass was positively correlated with laying order and the maximum temperature two days before egg laying, but was not predicted by female age; however, the assumption that heavier eggs are of higher quality may fail to account for the composition and concentrations of key resources for embryos in yolks. Embryonic viability increased with later clutch initiation dates, and was not significantly predicted by temperature, female age, egg mass, or laying order, and did not differ between study sites or years. Finally, I opportunistically examined maternal plasticity and prenatal reproductive success in response to a precipitous decrease in ambient temperature and associated rainfall (i.e., cold snap) during the egg laying period in one of the two years examined. Most females did not initiate incubation during the cold snap and nearly half of the females laying during the cold snap skipped at least one day of egg laying. While individual quality did not predict whether a female would have a laying skip, females with laying skips laid heavier eggs and larger clutches. Laying skips were not directly related to embryonic viability; however, laying skips and delayed incubation increase the lay-to-set interval of earlier-laid eggs, which may decrease hatching success. This study is the first to quantify fertility and developmental stages of embryonic mortality in tree swallows and provides opportunity for further investigation into factors contributing to hatching failure in aerial insectivores, especially as climate variability increases.

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Dedication

This thesis is dedicated in memory of my two grandmothers. My paternal grandmother, Faye, adored birds and would have been delighted see me become an ornithologist. My maternal grandmother, Lily, worked tirelessly to ensure her children and grandchildren could receive the education she didn’t have the opportunity to complete. I am forever grateful for everything she did so I could succeed.

Chapter 1: General introduction

Worldwide, many avian species are declining as the effects of large-scale, anthropogenic pressures continue to rise (Lees et al., 2022). Climates are changing at unprecedented rates, including increased variability in weather patterns (Maibach et al., 2014), which threatens individuals unable to respond effectively to sudden changes in local environmental conditions. These recent shifts in climate regimes are contributing to avian population declines, due in part to decreased foraging efficiency and habitat availability (reviewed in Jenouvrier, 2013). Population declines occur when mortality and emigration rates exceed birth and immigration rates, and embryonic mortality may be a potentially important driver of demographic processes in many animal populations (Chen et al., 2020). Whereas factors driving mortality in the nestling, fledgling, and adult life stages are well-studied in many avian species (e.g., Martin et al., 2018; Remeš, 2007), little is known about prenatal mortality in birds. Avian eggs are ideal for the study of the prenatal life stage as each egg is an external chamber that, provided adequate microclimatic conditions, contains all resources required for a single-celled zygote to develop into a fully formed hatchling (Carey, 1996).

Aerial insectivores, a guild comprised of swallows, swifts, flycatchers, and nightjars, are one of the fastest declining groups of birds in North America (Birds Canada & Environment and Climate Change Canada, 2024; Rosenberg et al., 2019). Their decline has been attributed to their sensitivity to climate change, as well as other changes in land use, pollution, and decreasing insect populations, which may affect all life stages, but the drivers of these declines remain equivocal (Nebel et al., 2010; Smith et al., 2015; Spiller & Dettmers, 2019). The extent to which each of these drivers might affect the prenatal life stage, and whether infertility or changes in fertility rates may be an additional factor contributing to population declines is currently unclear.

If an egg is fertile (i.e., a sperm cell successfully fused with an ovum to create a zygote), the probability of hatching success depends on maternal investment during egg formation and parental care during incubation, both of which are costly to adult birds (Nord & Williams, 2015; Williams, 2005). Across avian species, up to 17% of eggs on average do not hatch under normal conditions (reviews in Koenig, 1982, and Marshall et al., 2023). Many genetic and environmental factors can result in unhatched eggs, including inbreeding (Briskie & Mackintosh, 2004), congenital abnormalities (Liptói & Hidas, 2006), pollution (Fry, 1995), inadequate nutrition (e.g., Bidwell & Dawson, 2005), microbial infection (Cook et al., 2003), and unfavourable microclimatic conditions during incubation (Webb, 1987; Christensen, 2001). Since the factors contributing to hatching failure are so varied, it is important to investigate hatching failure within individual species or populations separately to infer if causes of hatching failure are driven by infertility or embryonic mortality. The extent to which hatching failure is a result of extrinsic (e.g., environmental conditions) or intrinsic (e.g., female age) factors is also important to determine for researchers to better understand the primary drivers of hatching failure within populations.

1.1 STUDY SPECIES AND STUDY AREA

Tree swallows (*Tachycineta bicolor*) are migratory aerial insectivores and secondary cavity nesters that breed across Canada and the northern United States in open, semi-treed habitat usually near water (Winkler et al., 2020a). Natural cavities used by tree swallows are typically those excavated by other avian species, often woodpeckers, but individuals also use naturally occurring hollows, such as broken tree limbs and tree stumps (Aitken & Martin, 2008), as well as human-provided nest boxes (Jones, 2003). The diet of tree swallows is composed of small flying insects caught in flight, such as dipterans, hemipterans, and coleopterans (Harris, 2023). Tree swallows are “income breeders”, rapidly using nutrients to form eggs shortly after consuming food (Jönsson, 1997). Insect abundance during the egg formation period has been associated positively with clutch size and egg mass, and negatively with the duration of incubation (Hussell & Quinney, 1985; Nooker et al., 2005). The key period of yolk formation (i.e., rapid yolk development; RYD) for tree swallows lasts five to six days, and egg mass is most influenced by insect abundance two to three days before an egg is laid (Ardia et al., 2006c); therefore, eggs are typically heavier and contain larger quantities or higher quality of embryonic nutrition when insect abundance is higher during the RYD period. Additionally, the abundance of flying insects is correlated with local weather conditions at the breeding sites of insectivorous birds (Cox et al., 2019; Grüebler et al., 2008). Consequently, eggs laid immediately following warmer and drier site conditions are expected to be heavier and contain higher quality yolks, resulting in higher hatching success. Female tree swallows lay one egg per day with clutch size usually ranging from 4 – 7 eggs and begin an incubation period of 13 to 14 days typically after the penultimate or final egg is laid (Zach, 1982). Females are solo intermittent incubators, providing the entirety of care to their eggs during incubation and leaving their nests multiple times per day for foraging bouts (Winkler et al., 2020a).

Tree swallows are socially monogamous, although they frequently engage in extra-pair copulations (Berzins & Dawson, 2020; Dunn & Whittingham, 2007). Adult males have dorsal plumage with a blue-green iridescence produced by the interaction between light waves and the nanostructure of the feathers (Prum, 2006). Whereas males grow adult plumage immediately following their first post-fledging molt, second-year females (who are in their first year of breeding) have delayed plumage maturation and display a high proportion of brown dorsal feathers before developing adult plumage as after-second-year females (Hussell, 1983).

I studied tree swallows breeding in nest boxes near Prince George, British Columbia, Canada (53° N, 123° W), in 2022 and 2023 at three established study sites – an agricultural site, a wetland site, and a mixed habitat site. This area in central British Columbia is in the Sub-Boreal Spruce biogeoclimatic zone, which is characterized by annual fluctuations in temperature extremes between summer and winter, and moderate precipitation (Meidinger et al., 1991). Nesting activity by tree swallows in this region typically begins in early May. Nest boxes at all sites were mounted ~1.5 m above ground on fence posts. The agricultural site contained 131 nest boxes approximately 30 m apart along fence lines in hay fields and along roads near grazing sites for cattle. The wetland site included 60 boxes distributed at approximately 15 – 20 m spacing around a managed wetland. The mixed habitat site contained 18 boxes on a single fence line between agricultural fields, with a large pond nearby.

1.2 GENERAL OBJECTIVES

1.2.1 Determining the fertility rate of tree swallows using advancements in avian fertility testing microscopy

Assessing demographic changes in populations requires an understanding of processes that contribute to mortality at all life stages of individuals, including prenatally (Evans & Postma, 2024; Grafen, 1988). To fully assess if hatching failure contributes to population declines, it is important to understand whether hatching failure is due to infertility (a sperm cell fails to fuse with the ovum) or embryonic mortality, as these causes are driven by different mechanisms. Infertility indicates a potential lack of viable sperm available to the ovum following ovulation, while the death of an embryo may be caused by a variety of extrinsic (e.g., environmental conditions) or intrinsic (e.g., genetic) factors. Additionally, mortality in the early, middle, and late stages of embryonic development can be linked to different factors (reviewed in Assersohn et al., 2021; Christensen, 2001); therefore, quantifying the time of death of inviable embryos can reveal patterns and causes of developmental failure within a population. Importantly, embryonic mortality is most common in early stages of development, often before embryos are visible macroscopically (Christensen, 2001); therefore, eggs containing embryos that died early in development may be easily mistaken as infertile. Microscopic methods to differentiate between early embryonic mortality and infertility, however, have been underused by researchers, resulting in an overestimation of infertility, or an inability to infer the causes of prenatal mortality in avian populations (Hemmings & Evans, 2020).

To my knowledge, the only previous study of fertility in tree swallows is Kempenaers et al. (1999), who examined eight eggs microscopically, which were subsequently assumed to be fertile due to the presence of sperm and sperm holes in the perivitelline layer of the yolk. Although the presence of sperm and sperm holes suggested the eggs did not hatch due to a lack of sperm, the detection of embryonic cells would have been required to conclusively confirm that sperm successfully fused with the ovum to create a fertilised egg. The microscopy test used by Kempenaers et al. (1999) was later modified to include identification of both sperm and embryonic nuclei in and around the germinal disc of the yolk (Birkhead et al., 2008). I used this newer microscopy technique in Chapter 2 with the goal of determining the fertility rate of tree swallows to better understand the underlying mechanisms that may lead to hatching failure in this species. I also quantified the proportions of embryos that died in early, middle, and late incubation to determine whether mortality varied among developmental stages. Additionally, I used a set of eggs collected in 2016 and subsequently frozen for eight years to determine if the efficacy of microscopic avian fertility testing was affected by long periods of freezing.

1.2.2 The influence of extrinsic and intrinsic factors on prenatal reproductive success

Rapid yolk deposition (RYD) begins when yolk precursors are synthesized from the liver and deposited in follicles in the ovary of the female in the form of proteins and lipids to create a mature yolk (Williams, 2012). In birds that lay one egg per day, such as tree swallows, the follicles that activate in a breeding season sequentially initiate RYD one day apart (Williams, 2012), with the rate of deposition reaching a maximum in the last half of the RYD period (Ojanen, 1983). Because RYD is asynchronous, eggs within a clutch may receive different nutrient loads, both in composition and abundance, depending on the environmental conditions during RYD of each individual egg. These conditions may therefore affect the quality and viability of an egg, particularly for income breeding birds like tree swallows (e.g., Ardia et al., 2010). Additionally, because female tree swallows are solo incubators, they are largely responsible for embryonic viability (i.e., survival of an embryo to hatching), and females in better condition could be expected to have higher hatching success.

The combined components of an egg represent the entirety of the nutrition provided for a growing embryo; therefore, the size of an egg may be an important indicator of prenatal quality. For example, larger yolks are associated with greater protein uptake by embryos

(Christians & Williams, 2001), and chicks hatched from larger eggs are heavier and more likely to fledge (Krist, 2011). The effect of egg mass on embryonic viability, however, remains unclear. Studies of domestic poultry indicate eggs that are very small or very large have lower hatching failure (reviewed in Narushin & Romanov, 2002), and some studies of wild birds have found positive relationships between egg mass and hatching success (e.g., Lombardo et al., 2021; Sanchez-Lafuente, 2004), while others have found no effect (e.g., Beissinger et al., 2005; Di Giovanni et al., 2022). Furthermore, a few previous studies of tree swallows have quantified hatching failure rates (e.g., Di Giovanni et al., 2022; Ghilain & Bélisle, 2008), but researchers have been limited to studying factors contributing to the general failure of eggs to hatch rather than embryonic mortality, as the fertility of tree swallows has been unknown. After quantifying rates of fertility and embryonic mortality of the tree swallows in my study population in Chapter 2, I was able to examine factors that specifically may constrain embryonic viability in the species. In Chapter 3, I investigated the effects of local temperature and female quality on two measures of prenatal reproductive success – embryonic viability and egg mass.

1.2.3 Responses of females to adverse weather conditions

Wildlife species that breed annually in specific seasons, such as tree swallows, rely on cyclical changes to ambient conditions that determine food availability and ultimately the energy balance required for successful reproduction (Bronson, 2009; Carey, 2009; Somveille et al., 2018). When these regular cycles are disrupted and local conditions abruptly and adversely change during the breeding season, individuals must respond quickly to avoid offspring mortality. Additionally, warming temperatures are leading to advanced timing of breeding, yielding potential mismatches between the nestling period and food abundance

(Dunn, 2019). Increased variability in weather patterns and the occurrence of extreme weather events adds a complicating factor to the advancement of the egg laying period, yet less is understood about the plasticity of prenatal maternal investment in response to variability in local environmental conditions.

While most passerines lay one egg every day from clutch initiation to clutch completion, laying skips can occur in which at least one day passes without an egg laid. Because many birds wait to start incubation until their clutches are complete or nearly complete (Clark & Wilson, 1985), laying skips extend the lay-to-set intervals (the number of days between when an egg is laid and when incubation begins) for the earlier-laid eggs in the clutch. Hatchability may decrease when the period at which eggs remain in the nest without incubation increases (Aldredge, 2017; Beissinger et al., 2005); therefore, species that delay incubation may experience higher hatching failure of earlier-laid eggs (e.g., Sockman, 2008), and this effect may be exacerbated if individuals have laying skips or further delay the onset of incubation past laying of the last egg in the clutch. Decreases in hatching success linked to long lay-to-set intervals have been attributed to prolonged exposure of eggs to suboptimal ambient temperatures. Conversely, delaying hatch through laying skips, delayed incubation, and longer incubation periods may be beneficial to realign the nestling period with peak food abundance in species advancing the timing of their breeding (Both & Visser, 2005; Cresswell & McCleery, 2003; Tomás, 2015), or may be necessary for breeding adults to replenish sufficient body fat reserves to provide adequate care to embryos during incubation, as incubation can be energetically costly (Nord & Williams, 2015). Little is known about the measures of female quality that may predict the occurrence of laying skips during periods of environmental stress, and whether delayed hatch is ultimately beneficial or detrimental to reproductive success.

In Chapter 4, I opportunistically studied a 63-hour severe weather event in May, 2023, in which ambient temperature precipitously decreased and was accompanied by rainfall following a period of seasonally warm and dry conditions. This harsh weather event occurred during the egg laying period, so I assessed the response of breeding females through delayed onset of incubation and laying skips and further investigated the causes and consequences of laying skips as they related to female quality and embryonic mortality.

1.3 SIGNIFICANCE

As aerial insectivores decline across North America, understanding the factors that drive mortality in every life stage is important, including prenatally. My thesis research addressed knowledge gaps in the rates of fertility and early, middle, and late-stage embryonic mortality in tree swallows, as well as factors that may influence prenatal reproductive success of the species. Additionally, I examined the effects of a cold weather event on the egg laying period, and the extent to which female birds may be able to adapt physiologically and behaviourally to such events, which may be especially relevant given the increasing variability of weather due to climate change (reviewed in Vázquez et al., 2015). From an applied perspective, I demonstrated that microscopic avian fertility testing techniques can be used on eggs that have been incubated for lengthy periods and those that have been kept frozen in long-term storage.

Chapter 2: Hatching failure in tree swallows is driven by embryonic mortality, not infertility

2.1 ABSTRACT

Aerial insectivores in North America have rapidly declined over the past half-century, and factors contributing to these declines are poorly understood. Egg development is a crucial stage in the life cycle of a bird; across species including aerial insectivores, up to 17% of eggs fail to hatch under normal conditions. Failure of eggs to hatch can be a consequence of either infertility or embryonic mortality; however, methods to distinguish between the two types of unhatched eggs have been underused by researchers. Aerial insectivores that use nest boxes, such as tree swallows (*Tachycineta bicolor*), are an ideal study species in which to investigate hatching failure, as nesting behaviour can be monitored closely throughout the breeding season. In 2022 and 2023, I monitored 1090 eggs from 191 nests at three study sites near Prince George, British Columbia. Using fluorescence microscopy to identify embryonic and sperm nuclei, I determined that 98.3% to 100% of the eggs were fertile, and most cases of hatching failure were therefore due to embryonic mortality, not infertility. Embryonic mortality was most likely to occur during early incubation, before development is usually macroscopically visible. I also examined 114 eggs from 17 clutches laid by tree swallows in 2016 and stored frozen for eight years, except for a brief thaw-freeze event in 2020 due to a freezer malfunction. Importantly, I successfully used fluorescence microscopy on this set of eggs, despite the conditions in which they were stored, and found that the fertility rate of this sample of eggs from 2016 was similarly close to 100%. This study is the first to quantify fertility and developmental stages of embryonic mortality in tree swallows and will provide

an opportunity for further investigation into factors contributing to hatching failure in aerial insectivores. Recent advancements in avian fertility testing offer a relatively simple method for determining fertility of unhatched eggs and could allow researchers to enhance their understanding of the reproductive biology of their respective study species.

2.1 INTRODUCTION

Understanding the processes that influence mortality at all life stages, including prenatally, is important to fully understand demographic changes in populations (Evans & Postma, 2024; Grafen, 1988). In birds, the prenatal period is a particularly critical life history stage because egg development can be influenced by parental investment and behaviour as well as environmental conditions. An unhatched egg represents a large energetic loss to females because of the costs of egg formation (Williams, 2005) and to parents involved in incubation (Nord & Williams, 2015). Accordingly, there should be strong selection against inviable eggs in avian populations, yet up to 17% of eggs, on average, do not hatch under normal conditions (reviews in Koenig, 1982, and Marshall et al., 2023). To fully assess if hatching failure contributes to population declines, understanding whether hatching rates are varying temporally is important, and if hatching failure is due to infertility (a sperm cell fails to fuse with the ovum) or embryonic mortality. Importantly, embryonic mortality is most common in early stages of development, often before embryos are macroscopically visible (Christensen, 2001), resulting in eggs containing embryos that died early in development being mistaken as being unfertilised. Microscopic methods to differentiate between early embryonic mortality and infertility, however, have been underused by researchers, resulting in an overestimation of infertility, or an inability to infer reasons for prenatal mortality in avian populations (Hemmings & Evans, 2020). Additionally, mortality in the early, middle, and late stages of embryonic development can be linked to different factors (reviewed in Assersohn et al., 2021; Christensen, 2001); therefore, quantifying the time of death of inviable embryos can reveal patterns and causes of developmental failure within a population.

Globally, half of avian species are in decline (Lees et al., 2022). Aerial insectivores, a guild comprised of swallows, swifts, flycatchers, and nightjars, are one of the fastest declining groups of birds in North America (Birds Canada & Environment and Climate Change Canada, 2024; Rosenberg et al., 2019). Declines in aerial insectivores have been attributed to their sensitivity to changes in land use, climate, pollution, and decreasing insect populations, which may affect all life stages, but the drivers of these declines remain equivocal (Nebel et al., 2010; Smith et al., 2015; Spiller & Dettmers, 2019). It is currently unclear to what extent each of these drivers might affect the prenatal life stage, and whether infertility or hatching failure rates may contribute to population declines.

Aerial insectivores that use nest boxes, such as tree swallows (*Tachycineta bicolor*), are ideal study species for investigations of hatching failure, as their reproductive activities can be monitored closely from initial nest building and clutch initiation to egg incubation and hatching. Whereas adult fitness and juvenile development are extensively studied at many breeding sites across North America (e.g., Berzins et al., 2020; Lombardo et al., 2021, Winkler et al., 2020b), less is known about the development of tree swallow eggs and why they may fail to hatch. Studies of reproductive success in tree swallows often do not report hatching failure rates (e.g., Lombardo, 1994; Lombardo et al., 2021; Nooker et al., 2005; Taylor & Cristol, 2015). Several studies of tree swallow populations have reported mean hatching failure rates, ranging from 8% to 23% (Bidwell & Dawson, 2005; Cox et al., 2018; Kempenaers et al., 1999; Stutchbury & Robertson, 1988); however, these studies did not specify if abandoned and depredated eggs were included in their respective estimates of hatching failure, making comparative analyses and detection of changes to these rates over time difficult (see also review in Marshall et al., 2023). To date, two studies have reported hatching failure rates of tree swallows with abandoned, broken, and depredated eggs clearly excluded: An analysis of the effect of agricultural intensification by Ghilain and Bélisle (2008) reported a rate of 14%, and a comparative study of 14 species by Di Giovanni et al. (2023) reported a hatching failure rate of 10.5% for tree swallows.

To my knowledge, the only previous study of fertility in tree swallows is that of Kempenaers et al. (1999), who collected 17 unbroken eggs for microscopic fertility testing. Nine of these eggs were not analyzed because they were too dry for analysis. The remaining eight eggs were assumed to be fertile due to the presence of sperm and sperm holes in the perivitelline layer of the yolk. While the presence of sperm and sperm holes suggests the eggs did not hatch due to a lack of sperm, the detection of embryonic cells would have been required to conclusively confirm that sperm successfully fused with the ovum to create a fertilised egg. The microscopy test used by Kempenaers et al. (1999) was subsequently modified to include identification of both sperm and embryonic nuclei in and around the germinal disc of the yolk (Birkhead et al., 2008). I used this newer microscopy technique with the goal of determining the fertility rate of tree swallows to better understand the underlying mechanisms leading to hatching failure in this species. I also quantified the proportions of embryos that died in early, middle, and late incubation to determine whether mortality varied among developmental stages.

2.2 MATERIALS AND METHODS

2.2.1 Study species, study area, and general field methods

Tree swallows are migratory aerial insectivores and secondary cavity nesters that breed across Canada and the northern United States in open, semi-treed habitat usually near water (Winkler et al., 2020a). Natural cavities used by tree swallows are typically those excavated by other avian species, often woodpeckers, but individuals also use naturally occurring hollows, such as broken tree limbs and tree stumps (Aitken & Martin, 2008), as well as human-provided nest boxes (Jones, 2003). Tree swallows are socially monogamous, though they frequently engage in extra-pair copulations (Berzins & Dawson, 2020; Dunn & Whittingham, 2007). Females lay one egg per day with clutch size usually ranging from 4 – 7 eggs and begin a 13 to 14-day incubation period after either the penultimate or final egg is laid (Zach, 1982). Females are solo intermittent incubators, providing the entirety of care to eggs during incubation, and leaving their nests multiple times per day for foraging bouts (Winkler et al., 2020a).

I studied tree swallows breeding in nest boxes near Prince George, British Columbia, Canada (53° N, 123° W), in 2022 and 2023 at three established study sites – an agricultural site, a wetland site, and a mixed habitat site (composed of farmland with a wetland adjacent to the nest boxes). This area in central British Columbia is in the Sub-Boreal Spruce biogeoclimatic zone, which is characterized by annual fluctuations in temperature extremes between summer and winter, and moderate precipitation (Meidinger et al., 1991). Nest boxes at all sites were mounted ~1.5 m above ground approximately 20 – 30 m apart on fence posts in hay fields, grazing pasture, and near wetlands. There were 131, 60, and 18 boxes at the agricultural, wetland, and mixed habitat sites, respectively.

Nest boxes were monitored at the agricultural and wetland sites in 2022, and at all three study sites in 2023. The boxes were emptied of all nesting material at the end of each breeding season. I began checking nest boxes every two days beginning in early May to monitor nest construction, and then daily throughout the nest building and egg laying stages. Eggs were sequentially numbered using a non-toxic pen on the day they were laid. A clutch was considered complete when incubation had started and three consecutive days had passed without a new egg being laid.

2.2.2 Viability assessment

I estimated the date each female started incubation by assessing the surface temperature of the eggs using a digital infrared thermometer (nearest 0.1 ºC), and noted if the female flushed from the nest box upon approach, as the surface temperature of eggs will cool when females leave the nest to forage (Ardia et al., 2009). Unattended eggs that were being incubated were therefore expected to be slightly cooler than eggs incubated by a female that flushed as I arrived at the box. Three or four days after incubation was estimated to have begun, all eggs in each clutch were candled to assess viability by shining a light on the blunt end to illuminate the contents of the egg. Based on the stages of embryo development initially described by Hamburger and Hamilton (1951) for domestic fowl (*Gallus gallus domesticus*), Hemmings and Birkhead (2016) determined that passerine eggs incubated for three to four days have a visible embryo and numerous blood vessels. As the developing heart is visible at these stages, a heartbeat may also be observed, and I evaluated each egg for these indications of a living embryo (Figure 2.1). If development in most eggs was faint or not visible (i.e., the estimation of the onset of incubation was too early), I re-candled the clutch on subsequent days until development in viable eggs was consistent with passerine eggs incubated for at least three days (Hemmings & Birkhead, 2016; Murray et al., 2013).

All eggs that did not contain a living embryo were considered inviable, collected from the nest, and frozen at -20 ºC for up to one year (2022) or refrigerated at 4 ºC for up to six months (2023) until fertility testing (details below). Because female tree swallows often begin incubating after laying their penultimate egg (Zach, 1982), the last-laid egg was always left in the nest for the entire incubation period even if an embryo was not visible, and later collected when nests were checked for hatching if it was inviable. There were no cases in which an incubating female was present, but no development was visible within the entire clutch.

Nests were left undisturbed for the remainder of incubation until 12 days after clutch completion when daily nest checks were then resumed to record hatching date and assess hatching success of the remaining eggs. Unhatched eggs were candled two days after others in the clutch had hatched, and eggs that failed at a later stage than those collected during the first viability assessment, as well as last-laid eggs lacking visible development, were collected. Nests that remained dormant of any activity for at least five days, including a lack of new eggs laid, incubation (i.e., eggs were consistently cold), or observed interest in the nestbox by any female, were assumed to be abandoned. One nest was considered abandoned after the female was found deceased in the nest cup and all eggs were cold and contained inviable embryos. In tree swallows, competition for nests sites can be intense and nest usurpation is common (Leffelaar & Robertson, 1985). In such cases, any eggs present are usually buried by the female taking over the nest box. Eggs in abandoned nests (n = 53 eggs from 15 nests) and buried nests (n = 13 eggs from 4 nests) were always candled to ensure they did not contain living embryos, and then subsequently collected.

2.2.3 Fertility testing

When embryonic development was not visible in inviable eggs that I collected, fertility testing was carried out using fluorescence microscopy (Assersohn et al., 2021; Birkhead et al., 2008). Frozen eggs were thawed at room temperature and these eggs, as well as those that had been refrigerated, were each carefully opened into separate petri dishes containing 0.01 M phosphate-buffered saline. Whenever possible, the germinal disc on the yolk was located, and both the outer and inner perivitelline layers around the germinal disc were removed and placed on a microscope slide. Contents from eggs that had dried out were rehydrated by soaking in phosphate-buffered saline for at least 20 minutes. In instances where the germinal disc or the perivitelline layers had degraded, all pieces of perivitelline layer that could be found were isolated and placed on a microscope slide. Hoechst 33342 dye (0.05 mg ml^{-1}; bisbenzimide H 33342 trihydrochloride, Sigma-Aldrich Solutions) was applied to each sample, and the microscope slide was placed in a dark drawer for 5 minutes to allow the dye to penetrate the cells. If DNA is present, Hoechst dye binds to adenine-thymine units and fluoresces bright blue (Bucevičius et al., 2018), thereby allowing embryonic and sperm nuclei to be visually identified under a fluorescent microscope.

Each sample was searched systematically for cells using a Nikon Eclipse FN1 microscope with fluorescent light at 100x – 200x magnification, and a BP 340 – 380 excitation filter and a LP 425 suppression filter. Because fertile avian eggs contain thousands of embryonic cells when they are laid (Watt et al., 1993), embryonic nuclei present in fertile germinal discs were usually easily located in large sheets or clumps (Figure 2.2). In samples where the germinal disc had degraded, single or small groups of embryonic nuclei would be scattered across pieces of perivitelline layer. In these cases, I prepared multiple slides and searched until either I located small groups of embryonic nuclei, indicating the egg had been fertilised, or until I had exhausted search efforts across at least four slides. I classified samples in the latter case as “inconclusive” if the sample contained at least a few scattered fluorescing cells that were of a shape and size consistent with embryonic nuclei. If a sample contained no cells resembling embryonic nuclei, I classified the sample as unfertilised.

Sperm nuclei stained with Hoechst dye are also visible under fluorescent light. Birds are polyspermic, requiring multiple sperm to penetrate the ovum to result in successful fertilization (Hemmings & Birkhead, 2015); therefore, numerous sperm cells were usually observed in fertile samples (Figure 2.2). The presence of embryonic nuclei, however, was always used as primary evidence that an inviable egg was fertile. An effort was made to ensure embryonic nuclei were not irregularly formed as this could be an indication of the rare phenomenon of facultative parthenogenesis (i.e., asexual reproduction by a female that usually reproduces sexually; Schut et al., 2008). No samples in my study contained cells that potentially indicated parthenogenesis. When degraded pieces of perivitelline layers were searched for cells, the combined presence of scattered embryonic and sperm nuclei was used as evidence of fertility.

2.2.4 Effects of long-term freezer storage on fertility testing

In 2016, 114 eggs were collected from full clutches laid in 17 nests boxes at the mixed habitat site as part of a different experiment. Eggs were collected one day after they were laid and frozen at -20 ºC. The eggs remained frozen until 2020 when a mechanical failure of the freezer was discovered during the early months of the coronavirus pandemic. Because laboratory personnel were working remotely due to protocols related to the pandemic, the freezer likely did not operate for several weeks, and its contents thawed to room temperature. When the failure of the freezer was discovered, the eggs were re-frozen at -20 °C until 2024. The history of this set of eggs provided a unique opportunity to assess if: 1) the same fertility testing technique could be used on eggs that have been frozen for many years and subjected to freeze-thaw-freeze conditions; and 2) fertility of tree swallows breeding near Prince George, British Columbia, has changed from 2016 to 2022 – 2023.

2.2.5 Developmental staging of inviable embryos

Unhatched eggs containing macroscopically visible embryos were dissected into 0.01 M phosphate-buffered saline. Embryos were staged using a guide for zebra finches (*Taeniopygia guttata*) developed by Murray et al. (2013) and adapted from Hamburger and Hamilton's (1951) original developmental staging series for the domestic fowl. Like tree swallows, zebra finches are an altricial passerine bird with an average incubation of 14 days (Murray et al., 2013), and provide a more relevant comparison than domestic fowl, which are precocial and incubate for 21 days. I divided the developmental stages, and associated days of development for a 14-day altricial incubation period, into three groups based on the biological processes that occur during avian embryo development: early incubation (Stages 1 – 24; days 0 – 5), middle incubation (Stages 25 – 41; days 5 – 10), and late incubation (Stages 42 – 45; days 10 – 14). Stages 1 – 24 comprise the primary period of cell differentiation and embryogenesis, and Stages 25 – 41 constitute embryonic growth (Hamburger & Hamilton, 1951). Stages 42 – 45 are associated with the peak of embryonic oxygen consumption that occurs when incubation is 80% complete and the embryo prepares for and engages in the hatching process (Murray et al., 2013; Tazawa & Whittow, 2000).

I used a chi-square test to assess whether the proportions of embryos that died in early, middle, and late incubation differed significantly, and performed pair-wise comparisons between categories with significance set at alpha = 0.05. Eggs that were abandoned (n = 67), broken (n = 37), or depredated (n = 81) were excluded from this analysis. In 2023, a portion of nests at the agricultural site were experimentally altered following incubation initiation for a separate study, and unhatched eggs from these clutches were also excluded from the chi-square test and pair-wise comparisons (n = 128).

2.3 RESULTS

2.3.1 Hatching failure and fertility rates of tree swallows

A total of 1090 eggs were monitored in 2022 and 2023, and 288 of these eggs (26.4%) failed to hatch. The hatching failure rate was 10.1% (75/739) after abandoned, broken, depredated, and late-laid eggs, as well as eggs from experimentally altered nests, were excluded. To evaluate fertility rates of this population, I excluded 32 eggs that were depredated or went missing (likely from predation events) before the first viability assessment; therefore, 1058 eggs were included in fertility rate calculations. Most eggs (954/1058; 90.2%) had visible embryonic development, including 802 eggs that hatched successfully and 152 eggs that did not hatch but contained a visible embryo (Table 2.1). The remaining 104 eggs (9.8%) failed to hatch and did not contain visible embryonic development. Nine of these eggs were discarded because they either contained fungal or bacterial growth or had broken and dried out over the course of incubation to a point where they could not be rehydrated for microscopy. A further nine eggs had inconclusive fertility results, meaning I found at least a few cells that were of a shape and size consistent with embryonic nuclei, but the cells were scattered and not grouped in large sheets or clumps. Of the remaining 86 undeveloped eggs, all (100%) were confirmed fertile via fluorescence microscopy (Table 2.1). The minimum fertility rate for all tree swallow eggs monitored in 2022 and 2023 (assuming all inconclusive and discarded eggs were unfertilised) was therefore 98.3%, and the maximum fertility rate (assuming all inconclusive and discarded eggs were fertilised) was 100% (Table 2.1).

2.3.2 Fertility of eggs laid in 2016

Almost all eggs (108/114) collected in 2016 at the mixed habitat site and stored in a freezer were confirmed fertile via fluorescence microscopy (94.7%), and the remaining eggs (6/114) were categorized as inconclusive, as the perivitelline layer was highly degraded but at least a few cells resembling embryonic nuclei were identified. Despite thawing for several weeks and subsequently re-freezing in 2020, all eggs were in good condition, and none were discarded. Since no eggs in this set were conclusively unfertilised, the maximum fertility rate (assuming all inconclusive eggs were fertilised) was 100%.

2.3.3 Developmental staging of inviable embryos

Of the 78 eggs that failed to hatch and were included in developmental staging analyses, six were discarded either due to poor condition or because the embryo may have died during early incubation, but fertility testing was inconclusive. The remaining 72 embryos were staged and divided into early, middle, and late incubation mortality groups (Table 2.2). The proportion of eggs experiencing embryonic mortality differed among developmental stages ($\chi^2 = 30.25$, df = 2, $P < 0.001$), with the majority of mortality occurring during early incubation (63.9%), and the remainder equally split between middle incubation (18.1%) and late incubation (18.1%). Pairwise comparisons between groups showed that the relative proportion was higher in the early stage group than the middle and late stages (both $\chi^2 = 18.46$, df = 1, $P < 0.001$).

2.3.4 Tables

Table 2.1. Fertility of 1090 eggs laid by tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada, in 2022 and 2023. Fertility of eggs lacking visible embryonic development was confirmed by staining samples of the germinal disc or perivitelline layers of the yolk with Hoechst 33342 fluorescent dye and observing embryonic and sperm nuclei at 100x – 200x magnification (see Materials and Methods for details). Samples with only a few single cells present that resembled embryonic nuclei were classified as "inconclusive", and samples that were too degraded for microscopy or contained fungal or bacterial growth were discarded. The fertility status of eggs that were depredated or went missing before a candling viability assessment could not be determined. Minimum fertility is calculated as (([eggs with visible embryonic development] + [eggs confirmed fertile via fluorescence microscopy]) / ([total eggs laid] – [eggs depredated or missing before viability assessment]) * 100%), and maximum fertility is calculated as (([total eggs laid] – [eggs depredated or missing before viability assessment] – [unfertilised eggs]) / ([total eggs laid] – [eggs depredated or missing before viability assessment]) * 100%).

Site	Total eggs laid	Depredated or missing before viability assessment	Visible embryonic development	Confirmed fertile via fluorescence microscopy	Inconclusive	Discarded	Unfertilised	Minimum fertility (%)	Maximum fertility (%)
2022									
Stewards	223	5	196	18	4	0	0	98.2	100
Western	252	3	233	12	3	1	0	98.4	100
2023									
Stewards	317	7	287	18	2	3	0	98.4	100
Western	210	17	160	29	0	4	0	97.9	100
Dykes	88	0	78	9	0	1	0	98.9	100
Total	**1090**	**32**	**954**	**86**	**9**	**9**	**0**	**98.3**	**100**

Table 2.2. Percentages of embryos that died in the early (Stages 1 – 24), middle (Stages 25 – 41), and late (Stages 42 – 45) stages of incubation from tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada, in 2022 and 2023. Developmental stages are based on the staging guide developed by Murray et al. (2013) and adapted from Hamburger and Hamilton (1951).

Site	n	Early (%)	Middle (%)	Late (%)
2022				
Agricultural	21	61.9	14.3	23.8
Wetland	17	52.9	17.6	29.4
2023				
Agricultural	7	28.6	71.4	0.0
Wetland	19	89.5	0.0	10.5
Mixed habitat	8	62.5	25.0	12.5
Total	**72**	**63.9**	**18.1**	**18.1**

2.3.5 Figures

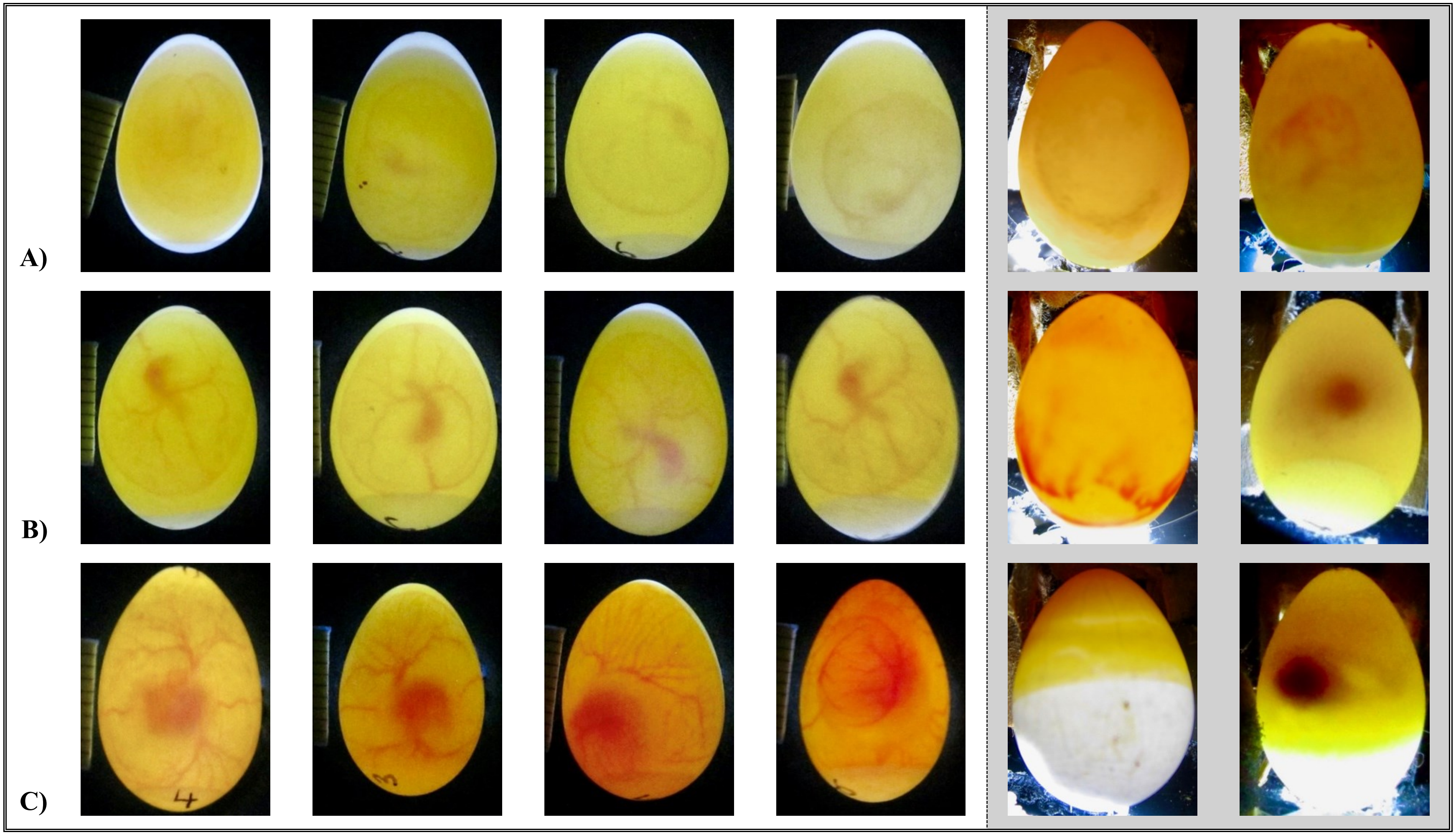


Figure 2.1. Photographs of viable (left four columns) and inviable (right two columns) eggs laid by tree swallows (*Tachycineta bicolor*) in central British Columbia, Canada. Eggs were candled three days (A), four days (B), and five days (C) after incubation was initiated.

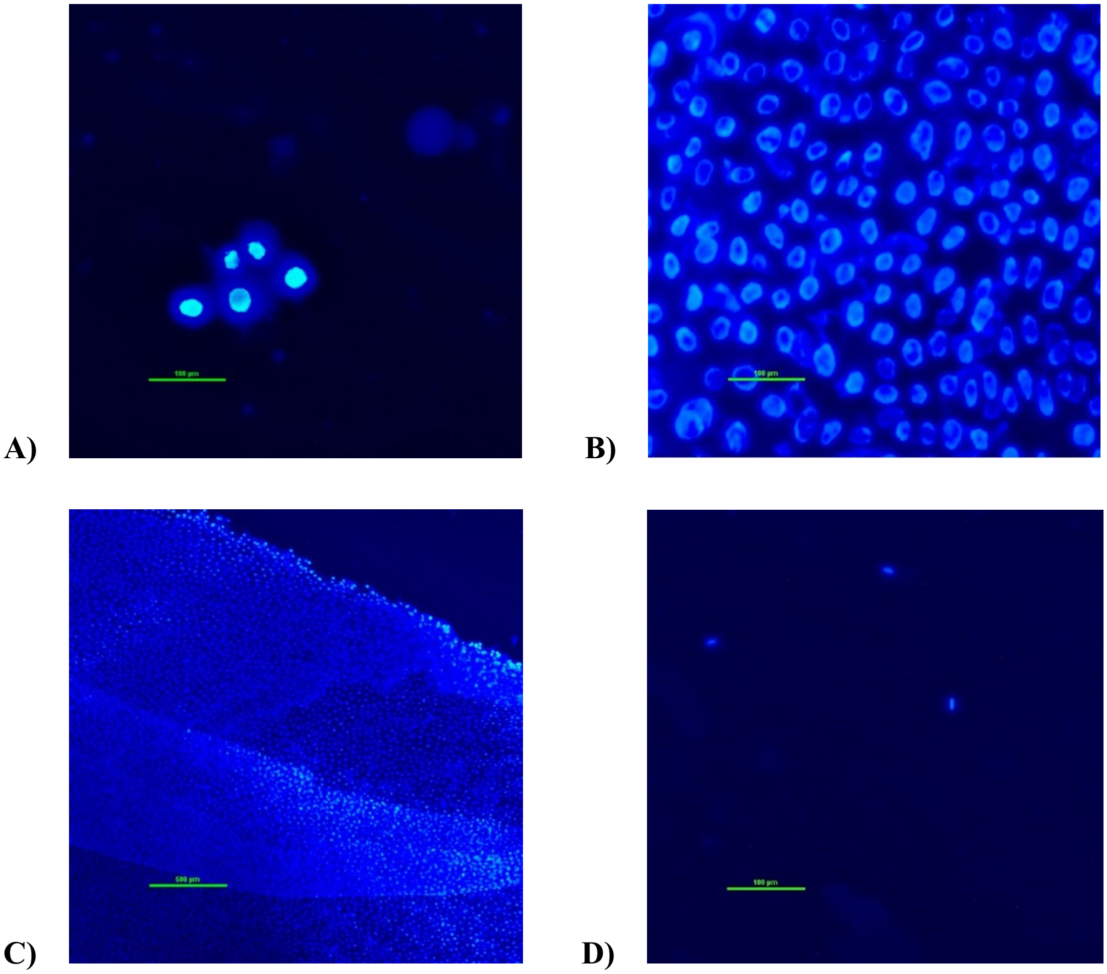


Figure 2.2. Photographs of samples from the germinal discs and surrounding perivitelline layers of unhatched eggs laid by tree swallows (*Tachycineta bicolor*). Embryonic nuclei were typically detected in small clumps (A) when the perivitelline layers had disintegrated, and sheets (B and C) when the yolk was relatively intact. Sperm nuclei (D) were found between the inner and outer perivitelline layers. Cells were dyed with Hoechst 33342 stain and illuminated under fluorescent light at 100x magnification (C) or 200x magnification (A, B, D). The presence of embryonic and sperm cells confirms these eggs were fertilised.

2.4 DISCUSSION

Of all eggs that required microscopic fertility testing from tree swallows within this study population, I found that no eggs were conclusively categorized as unfertilised, resulting in a maximum fertility rate of 100%, assuming discarded and inconclusive eggs were fertile. If discarded and inconclusive eggs were unfertilised, the minimum fertility rate would be 98.3%; however, this is unlikely. All eggs categorized as inconclusive had degraded perivitelline membranes and samples contained at least a few cells resembling embryonic nuclei but were conservatively labelled as inconclusive because the cells were scattered and not arranged in the groups typical of a conclusively fertile sample. Furthermore, there is no biological reason that the eggs that were discarded due to higher levels of degradation or fungal or bacterial growth would be more likely to be unfertilised. It is therefore probable that the fertility rate of tree swallows within this study population is 100%.

This study is the first to my knowledge to quantify fertility and developmental stages of embryonic mortality in tree swallows. The extremely high fertility rate of eggs in my study is consistent with fertility rates in other widely studied passerines: the great tit (*Parus major*), blue tit (*Cyanistes caeruleus*), house sparrow (*Passer domesticus*), and Eurasian tree sparrow (*Passer montanus*; Birkhead et al., 1995; Birkhead et al., 2008; Hemmings & Evans, 2020; Kato et al., 2017). Fertility rates of endangered avian species are also relatively high (Hemmings et al., 2012; Morland et al., 2024). Hatching failure in these populations and mine, and likely many others, is therefore predominantly due to embryonic mortality, not infertility. Future studies in tree swallows and other birds, especially those at established sites intended for long-term studies, should incorporate prenatal mortality into demographic data to ensure adequate representation of this age class in determining lifetime reproductive success and other similar demographic metrics. Additionally, the primary drivers of embryonic mortality should be assessed to further our understanding of the susceptibility of embryos to changes in climatic and other conditions.

Whereas hatching failure in aerial insectivores has not been identified as a driver of the recent declines in this guild across North America (Cox et al., 2018), establishing baseline data for each life stage of a population or species would help predict future variability in these metrics. Basic demographic data, like birth rates (i.e., hatching rates in birds), can also inform species conservation policies, such as management of individuals in zoos and captive breeding facilities (Conde et al., 2019). Additionally, the type of samples included in fertility data, such as abandoned, broken, and depredated eggs, should be clearly reported for accurate comparisons among populations or species, or comparisons between time periods. In the case of hatching failure in tree swallows, researchers previously did not report whether specific types of hatching failure were included in analyses. For example, Cox et al. (2018) reported hatching failure ranging from approximately 5% to 50% over 43 years, but it is unclear if the years with higher hatching failure were associated with large depredation events, disturbance from researchers, or similar external drivers. This is not exclusive to studies of tree swallows. In a review of hatching failure rates reported in studies of free-living birds, Marshall et al. (2023) excluded 24 studies in which the definitions of the reported hatching success or failure rates were either unclear or did not match the definition used by the review authors. Removing abandoned, broken, and depredated eggs from this present study yielded a hatching failure rate of 10.1%, consistent with hatching failure rates observed in many other avian populations in which abandoned, depredated, and broken eggs were also excluded (Koenig, 1982; Marshall et al., 2023). Importantly, even abandoned eggs were all fertile, suggesting a lack of viable sperm or copulatory events are not causes of abandonment in this population.

Most embryonic mortality in this study occurred during early incubation, which is common across many avian species (Assersohn et al., 2021; Christensen 2001; Hemmings & Evans, 2020), as well as many other vertebrates (Chen et al., 2020). This finding highlights the importance of microscopic fertility testing to differentiate between infertility and early embryonic mortality. In this study, 8.13% (86/1058) of eggs would have been assumed infertile without fertility testing.

Unexpectedly, incidences of mortality during middle and late incubation were similar. This is inconsistent with previous observations in other avian species that indicate mortality associated with late incubation is generally higher than mortality associated with the middle stages of incubation (Assersohn et al., 2021; Romanoff, 1972). While many factors have been linked to early-stage and late-stage embryonic mortality, little is known about the potential causes of mid-incubation mortality and why death during this period of incubation is relatively uncommon. (Assersohn et al., 2021; Christensen, 2001). The relative proportion of mortality during middle incubation was higher at the agricultural and mixed habitat sites in 2023 than either of the sites sampled in 2022, and there was no embryonic mortality during middle incubation at the wetland site in 2023. It is unclear if these differences between sites and years occurred by chance or are due to differences in site-specific conditions. Longer-term data are needed to establish if the patterns seen in this study are consistent with embryonic mortality of tree swallows across North America, and this study provides opportunity for further investigation into factors contributing to hatching failure in aerial insectivores.

2.4.1 Advancements in avian fertility testing

The goal of microscopic avian fertility testing was previously to identify the presence of sperm nuclei and sperm holes in the perivitelline layers of the yolk (e.g., Kempenaers et al., 1999; Small et al., 2000). Birkhead et al. (2008) then determined fluorescence microscopy could also be used to detect the presence of embryonic nuclei in the germinal disc, with Assersohn et al. (2021) updating and refining these methods. This advancement in avian fertility testing was crucial because while the presence of numerous sperm indicate fertilization is likely, the successful formation of a zygote cannot be confirmed without visualization of embryonic nuclei. Despite this advancement, studies of avian fertility have continued to prioritize detection of sperm nuclei, likely because these studies also aim to quantify the number of sperm present (e.g., Croyle et al., 2015; Hemmings et al., 2012).

As research in avian fertility grows, individuals seeking to quantify fertility in their study populations or captive breeding programs should prioritize detection of both embryonic and sperm nuclei. Researchers should be cautious when relying solely on the presence of embryonic nuclei, as facultative parthenogenesis (i.e., asexual reproduction by a female that usually reproduces sexually) can occur in birds. To date, parthenogenesis has mostly been documented in domestic poultry (reviewed by Ramachandran & McDaniel, 2018), captive populations of zebra finches (Schut et al., 2008), and California condors (*Gymnogyps californianus*; Ryder et al., 2021). The phenomenon, however, is not well understood in wild bird populations. Schut et al. (2008) observed embryonic nuclei originating from parthenogenesis were irregularly formed in comparison to sexually produced embryonic nuclei, so efforts should be made to locate numerous regular and uniform embryonic nuclei before declaring a sample as fertile (see Birkhead et al., 2008 for a comparative description).

Importantly, even if the germinal disc was no longer visible following prolonged incubation or frozen conditions, I found that preparing multiple slides of disintegrated pieces of perivitelline layer for microscopy usually resulted in finding disintegrated portions of germinal disc as well. Thousands of embryonic cells are present in a fertile egg at oviposition (Watt et al., 1993) and could be successfully detected using a fluorescent stain even if the embryo died early in incubation and the egg was subsequently incubated for more than two weeks, as was the case with some of the eggs tested in this study.

Although Birkhead et al. (2008) and Assersohn et al. (2021) recommend storing unhatched eggs in cool storage for a maximum of one month, I demonstrated that eggs from tree swallows can be successfully tested for fertility after six months of refrigeration storage and eight years of frozen storage. Additionally, temporarily thawing eggs stored in a freezer did not decrease the efficacy of fluorescence microscopy for assessing fertility. Morland et al. (2024) also conducted fertility testing on eggs laid by hihi (*Notiomystis cinta*) collected after clutches had hatched (i.e., incubated at least 17 days) and eggs were frozen in long-term storage. While a proportion of the eggs used in my study were stored in a refrigerator for six months without fungal or bacterial growth, future research should prioritize frozen storage to minimize this risk if eggs need to be stored prior to testing.

As many avian species continue to decline globally (Lees et al., 2022), it is becoming increasingly important to understand the drivers of mortality in each life stage. To date, few ornithological studies have quantified fertility or prenatal mortality rates, likely in part due to the difficulty of distinguishing between infertility and early embryonic mortality. Although some species, such as tree swallows, may not be declining due to problems with infertility, it is vital to collect baseline data so changes in fertility rates can be detected in the future.

Recent advancements in avian fertility testing provide a relatively simple method for determining fertility and should be considered by researchers and conservationists seeking to better understand the reproductive biology of their respective study species. Knowledge of the fertility rates and drivers for embryonic mortality in populations of any declining or endangered avian species can subsequently be used to inform conservation management strategies, such as the use of artificial incubation and strategic decisions regarding mate-pairing and wild releases in captive breeding programs (e.g., Bussolini et al., 2023; Digby et al., 2023).

Chapter 3: Do environmental factors and female quality influence embryonic viability or egg mass in tree swallows?

3.1 ABSTRACT

Factors driving mortality in the nestling, fledgling, and adult life stages are well-studied in many avian species, yet little is known about prenatal mortality in birds. Across all avian species, up to 17% of eggs on average do not hatch, representing a substantial loss of maternal prenatal investment. Factors contributing to hatching failure vary widely, so it is important to investigate if causes of hatching failure are driven by infertility or embryonic mortality, as well as the extent to which hatching failure is a result of extrinsic (e.g., environmental conditions) or intrinsic (e.g., female age) factors. I investigated prenatal reproductive investment in a population of tree swallows (*Tachycineta bicolor*) in which eggs were confirmed to be fertile. In 2022 and 2023, I monitored 653 eggs from 113 nests at a wetland site and an agricultural site near Prince George, British Columbia. I assessed the effects of relative clutch initiation date, female age, the maximum temperature two days before egg laying, laying order, and habitat type on egg mass, and the effects of these same factors along with egg mass on embryonic viability (i.e., the embryo survived to hatching). Overall, 9.80% (64/653) of embryos failed to hatch, and the odds of an embryo surviving to hatching increased with later clutch initiation dates. Embryonic viability was not significantly predicted by temperature, female age, egg mass, or laying order, and did not differ between sites or years. Egg mass was positively associated with the maximum temperature two days before egg laying and laying order, but was not predicted by female age; however, the assumption that heavier eggs are of higher quality may fail to account for the composition

and concentrations of key resources for embryos in yolks. Although females that lay eggs earlier in the breeding season are qualitatively superior, embryonic development may be deleteriously affected by colder temperatures during this time period, either as a consequence of decreased egg content quality from a lower abundance of insect prey, or inferior incubation conditions. There may also be a trade-off between hatching success and juvenile survival, as earlier-breeding adults fledge more young. A dataset containing a larger number of embryos that died in middle and late stages is required to fully evaluate the effects of environmental conditions and maternal investment during the incubation period. Conversely, associations between the variables I studied can vary annually in tree swallows, so larger datasets with longer time frames should be used in future studies to continue to examine prenatal reproductive success in the species. This study provides a baseline for gaining insights into prenatal reproductive success of an aerial insectivore.

3.2 INTRODUCTION

Worldwide, many avian species are declining as the effects of large-scale pressures, such as climate change and habitat degradation, continue to rise (Lees et al., 2022). While factors driving mortality in the nestling, fledgling, and adult life stages are well-studied in many avian species (e.g., Martin et al., 2018; Remeš, 2007), little is known about prenatal mortality in birds, despite the potential importance of embryonic mortality in driving demographic processes in animal populations (Chen et al., 2020). Avian eggs are ideal for the study of the prenatal life stage, as each egg is an external chamber that, provided adequate microclimatic conditions, contains all resources required for a single-celled zygote to develop into a fully formed hatchling (Carey, 1996).

If an egg is fertile (i.e., a sperm cell successfully fused with an ovum to create a zygote), the likelihood of hatching success depends on maternal investment during egg formation and parental care during incubation, both of which are costly to adult birds (Nord & Williams, 2015; Williams, 2005). Across all avian species, up to an average of 17% of eggs in the wild do not hatch (reviews in Koenig, 1982, and Marshall et al., 2023). Many genetic and environmental factors can result in unhatched eggs, including inbreeding (Briskie & Mackintosh, 2004), congenital abnormalities (Liptói & Hidas, 2006), pollution (Fry, 1995), inadequate nutrition (e.g., Bidwell & Dawson, 2005), microbial infection (Cook et al., 2003), and unfavourable microclimatic conditions during incubation (Webb, 1987; Christensen, 2001). Because the factors contributing to hatching failure are so varied, investigations of hatching failure within individual species or populations separately could identify whether causes of hatching failure are driven by infertility or embryonic mortality, and the extent to which hatching failure is a result of extrinsic (e.g., environmental conditions) or intrinsic (e.g., female age) factors.

Avian egg formation is initiated prior to egg laying during a period called rapid yolk deposition (RYD), in which yolk precursors are synthesized from the liver and deposited in follicles of the ovary of the female in the form of proteins and lipids to create a mature yolk (Williams, 2012). In birds that lay one egg per day, the follicles that activate in a breeding season sequentially initiate RYD one day apart (Williams, 2012), with the rate of deposition reaching a maximum in the last half of the RYD period (Ojanen, 1983). Because RYD is asynchronous, eggs within a clutch may receive different nutrient loads, both in composition and abundance, depending on the environmental conditions during RYD of each individual egg. These conditions may therefore affect the quality and viability of an egg (e.g., Ardia et al., 2010). The combined components of an egg represent the entirety of the nutrition provided for a growing embryo; therefore, the size of an egg may be an important indicator of quality. For example, larger yolks are associated with greater protein uptake by embryos (Christians & Williams, 2001), and chicks hatched from larger eggs are heavier and more likely to fledge (Krist, 2011). The effect of egg mass on embryonic viability, however, remains unclear. Studies of domestic poultry indicate eggs that are very small or very large have lower hatching failure (reviewed in Narushin & Romanov, 2002), whereas studies on wild birds have found positive relationships between egg mass and hatching success (e.g., Lombardo et al., 2021; Sanchez-Lafuente, 2004), while still others have found no effect (e.g., Beissinger et al., 2005; Di Giovanni et al., 2022).

I investigated hatching failure in tree swallows (*Tachycineta bicolor*), a widely studied passerine bird distributed across North America (Winkler et al., 2020a). A few previous studies of tree swallows have quantified hatching failure rates (e.g., Di Giovanni et al., 2022; Ghilain & Bélisle, 2008), but researchers have been limited to studying factors contributing to the general failure of eggs to hatch rather than embryonic mortality, as the fertility of tree swallows was unknown (but see Kempenaers et al., 1999). I was able to examine factors that specifically constrain embryonic viability in the species because I found that hatching failure in the population of tree swallows I studied is due to embryonic mortality and not infertility (Chapter 2).

Tree swallows are migratory aerial insectivores and secondary cavity nesters that breed across Canada and the northern United States in open, semi-treed habitat usually near water (Winkler et al., 2020a). Their diet is composed of small flying insects caught in flight, such as dipterans, hemipterans, and coleopterans (Harris, 2023). Tree swallows are “income breeders", rapidly using nutrients to form eggs shortly after consuming food (Jönsson, 1997), and insect abundance during the egg formation period has been positively correlated with clutch size, egg mass, and duration of incubation (Hussell & Quinney, 1985; Nooker et al., 2005). The RYD period of tree swallows is five to six days and egg mass is most influenced by insect abundance two to three days before an egg is laid (Ardia et al., 2006c); therefore, eggs are likely heavier and contain superior embryonic nutrition when insect abundance is higher during the RYD period. Additionally, the abundance of flying insects is directly correlated with local weather conditions at the breeding sites of insectivorous birds (Cox et al., 2019; Grüebler et al., 2008), so eggs laid immediately following warmer and drier site conditions are likely to be heavier and contain higher quality yolks, resulting in higher hatching success.

Female tree swallows lay one egg per day with clutch size usually ranging from 4 – 7 eggs and begin a 13 to 14-day incubation period after either the penultimate or final egg is laid (Zach, 1982). Females are solo intermittent incubators, providing the entirety of care to their eggs during incubation and leaving their nests multiple times per day for foraging bouts (Winkler et al., 2020a). Other than abnormalities that may arise from the male's genetic contribution, female tree swallows are therefore largely responsible for embryonic viability, and females in better condition could be expected to hatch more eggs. The relative date that female tree swallows initiate egg laying is highly correlated with their quality. Females that lay earlier in the breeding season have longer wings (Winkler & Allen, 1996), stronger immune responses (Ardia, 2005; Hasselquist et al., 2001), lay larger eggs (Ardia et al., 2006a), lay larger clutches (Dunn et al., 2011; Stutchbury & Robertson, 1988), and produce more fledglings (Dunn et al., 2011). Additionally, earlier-laying females are better able to cope with experimentally inflated brood sizes (Ardia et al., 2003), and females with experimentally clipped wing feathers (and therefore decreased foraging efficiency) delay clutch initiation (Dunn & Whittingham, 2007).

Others measures of female quality in tree swallows include feather colour and female age. Adult tree swallows have dorsal plumage with a blue-green iridescence produced by the interaction between light waves and the nanostructure of the feathers (Prum, 2006). While males grow adult plumage following their first post-fledging molt, second-year (SY) females (who are in their first year of breeding) have delayed plumage maturation and display a high proportion of brown dorsal feathers before developing adult plumage as after-second-year (ASY) females (Hussell, 1983). This retention of juvenile colouring is thought to be a strategy to signal inexperience and reduce conspecific aggression from ASY females (Beck & Hopkins, 2019; Coady & Dawson, 2013). In addition to inexperience in breeding, SY females generally have lower reproductive success than ASY females (Ardia et al., 2006c; Beck et al., 2015; Bentz & Siefferman, 2013; Bitton & Dawson, 2017; Winker & Allen, 1996). Within the ASY age class, females with brighter and bluer feathers produce heavier eggs (Bitton et al., 2008; Bentz & Siefferman, 2013).

In this study, I investigated the effects of local temperature and female quality on prenatal measures of reproductive success in tree swallows breeding at a wetland site and an agricultural site. I did not investigate the effects of male quality, as tree swallows frequently engage in extra-pair copulations (Barber et al., 1996; Berzins & Dawson, 2020; Dunn & Whittingham, 2007) and I did not analyze the genetics of my study population. I used the date of clutch initiation as a measure of female quality, and I predicted that females who were older, more ornamented, and initiated egg laying earlier would lay larger eggs and experience less embryonic mortality within their clutches. I also predicted that eggs laid immediately following warmer days would contain embryos more likely to survive to hatch, as food availability for tree swallows (i.e., flying insects) is generally higher at warmer temperatures (Dunn et al., 2023).

3.3 MATERIALS AND METHODS

3.3.1 Study area and general field methods

I studied tree swallows breeding in nest boxes near Prince George, British Columbia, Canada (53° N, 123° W), in 2022 and 2023 at two established study sites – an agricultural site and a wetland site. This area in central British Columbia is in the Sub-Boreal Spruce biogeoclimatic zone, which is characterized by annual fluctuations in temperature extremes between summer and winter, and moderate precipitation (Meidinger et al., 1991). Nest boxes at both sites were mounted ~1.5 m above ground approximately 20 – 30 m apart on fence posts. The agricultural site contained 131 nest boxes distributed in hay fields and along roads near gazing sites for cattle. The wetland site contained 60 nest boxes arranged around a managed wetland. Local temperature (ºC), precipitation (mm), and windspeed (km/h) data were collected at each study site with weather stations (Onset Computer Corp.) in one-minute intervals throughout the breeding season. Additionally, I measured prey abundance using passive aerial insect samplers deployed to each site (Quinney & Ankney, 1985). Data collected by the insect samplers was ultimately not informative for my study (see Appendix A), and there was overall minimal precipitation and wind during the 2022 and 2023 breeding seasons. I therefore used daily maximum temperature as an indicator of insect abundance (Garrett et al., 2022).

Nest boxes were emptied of all nesting material at the end of each breeding season. I began checking nest boxes every two days beginning in early May to monitor nest construction, and then daily throughout the nest building and egg laying stages from mid-May to mid-June. Eggs were sequentially numbered using a non-toxic pen on the day they were laid and weighed with a digital scale (nearest 0.01 g) one day after they were laid. A clutch was considered complete when eggs were warm (i.e., incubation had started) and three consecutive days had passed without a new egg being laid. The relative clutch initiation date for each female was assigned by calculating the number of days between when the female laid her first egg and one day prior to the first egg laid in the breeding season at either site in each year (i.e., which had a relative hatch date of 1).

Once hatching commenced, I trapped females in their nest boxes as they entered to feed nestlings. Trapping usually occurred two days after at least one egg had hatched but was occasionally delayed due to cold or rainy weather. The minimum age of each female (n = 111) was determined based on plumage colouration and previous banding information. Nine females, who collectively laid 54 eggs, known to be a minimum of five or six years old were conservatively assigned a minimum age of four to decrease the risk of an outlier effect in analyses from the older ages. Abandoned, depredated, and broken eggs were excluded from analysis, as well as eggs laid very late in the breeding season that were likely renesting attempts following earlier breeding failure (Rooneem & Robertson 1997). In 2023, a portion of nests at the agricultural site were experimentally altered following incubation initiation for a separate study, and eggs from these clutches were also excluded from analyses. I collected and assessed the fertility status of all remaining unhatched eggs and determined no eggs included in the study were infertile (Chapter 2).

3.3.2 Feather colour analysis

During banding and measuring of each female, I collected a minimum of four rump feathers for spectral analysis and stored each set of feathers in a manila envelope at room temperature. Because SY females have delayed plumage maturation, I only included ASY females (n = 64) in feather colour analyses. To process the feathers, I arranged four feathers so that the shafts were taped to a piece of black cardboard and the feathers partially overlapped each other to mimic the natural arrangement of rump feathers on an individual tree swallow (Bitton et al., 2007). I placed each sample on a black background and measured colour spectra using a USB2000 spectrometer and a deuterium-halogen light source (Avantes, Apeldoorn, The Netherlands). Using a bifurcated probe in a black holder that excluded ambient light and held at 90º to the surface of the feathers, I took three measurements per sample, resetting the probe between measurements. I recorded spectral reflectance data using OOIBase32 software (Ocean Optics, Orlando, Florida) and calculated reflectance following Berzins & Dawson (2016).

I completed spectral analysis using the “pavo” package (Maia et al., 2019) in R version 4.3.1 (R Development Core Team, 2023). I smoothed the spectral curves and quantified the mean of each set of three measurements for four reflectance characteristics that are demonstrated to indicate the overall quality of ASY female tree swallows (e.g., Bitton et al., 2008): brightness (mean reflectance between 300 – 700 nm), hue (the wavelength of maximum reflectance), ultraviolet (UV) chroma (the proportion of light reflected from 300 – 400 nm relative to the entire spectrum), and blue chroma (the proportion of light reflected from 400 – 512 nm relative to the entire spectrum). Using the ASY females included in my study, I completed a principal component analysis to combine the four colour characteristics. The first principal component explained 59% of the total variation and was weighted by greater UV chroma and blue chroma; therefore, higher scores were associated with colour reflected at shorter wavelengths and were bluer (factor loadings: brightness = 0.17, UV chroma = 0.55, blue chroma = 0.53, hue = -0.63). The second principal component explained 32% of the total variation and was weighted by brightness (factor loadings: brightness = 0.81, UV chroma = 0.36, blue chroma = -0.41, hue = 0.20). Preliminary results showed feather colour was not a significant predictor in any analyses and was therefore not considered any further.

3.3.3 Statistical analyses

All statistical analyses were conducted in R version 4.3.1 (R Development Core Team, 2023). I used the “climwin” package (Bailey & van de Pol, 2016; van de Pol et al., 2016) to assess the relationship between temperature during the egg formation period and the mass of eggs laid by tree swallows during my study period. The “climwin” package uses Akaike Information Criterion scores corrected for small sample sizes (AIC_c) to compare the fit of regressions between a biological response variable and an environmental predictor variable over different time windows. If one or more regressions fit better than a null model, the package will assist in identifying time periods where the biological response variable is most sensitive to the environmental predictor variable. Within the “slidingwin” function in this package, I used generalized linear mixed effects models fit with the “lme4” package (Bates et al., 2015) and included female identity as a random effect. I tested the quadratic relationship, fit using a quadratic term in a linear model, between maximum daily temperature and egg mass for all possible daily time windows within 1 – 7 days before each egg was laid, as maximum daily temperature is quadratically related to the abundance of insect prey for tree swallows (Garrett et al., 2022). The most parsimonious model had maximum daily temperature that both started and ended two days before egg laying (AIC_c weight = 0.99); therefore, the maximum daily temperature two days prior to the date each egg was laid (max temp T-2) was used in analyses.

I used Student's t-tests to compare differences in temperature between study sites and between years, using Levene's tests to confirm variances were equal. For all other analyses, I used generalized linear mixed effects models fit using the "lme4" package (Bates et al., 2015), with P-values generated by the "lmerTest" package (Kuznetsova et al., 2017). To assess the effects of predictor variables on viability, eggs were assigned a binomial status of hatched (1) or failed to hatch (0), and binomial logistic regressions with a logit link function were used. Female identity was included as a random effect in all models. I determined generalized variance inflation factor values for the saturated models were < 3 (i.e., low collinearity among predictor variables) using the "car" package (Fox & Weisberg, 2019). Significance was set at alpha = 0.05, and all means and regression coefficients (β) are reported ± 1 standard error. A summary of the predictor variables measured in this study is provided in Appendix B.

I performed analyses to assess the nature of the relationships between egg mass and embryonic viability, and max temp T-2 and embryonic viability. Whereas the quadratic relationship between egg mass and viability was initially significant ($\beta = -0.16 \pm 0.08$, $z = -2.13$, $P = 0.03$), removing the smallest egg and largest egg from the data set yielded a non-significant result ($\beta = -0.07 \pm 0.11$, $z = -0.67$, $P = 0.50$; Figure 3.1). I therefore used a linear term for egg mass in all analyses and retained the smallest and largest eggs in the data set. I also used a linear term for max temp T-2, as the quadratic term did not explain any more variation in the data than the linear term. I assessed the effects of relative clutch initiation date, female age, max temp T-2, laying order, and site type (wetland or agricultural) on egg mass, and the effects of these same factors along with egg mass as a predictor variable on embryonic viability. Year was included as a covariate in both model sets. I used a backward stepwise elimination procedure to remove variables that did not approach significance ($P > 0.10$). I used the "DHARMa" package (Hartig, 2024) on the saturated models to ensure models passed normality, dispersion, and outlier tests.

3.4 RESULTS

During the 2022 and 2023 breeding seasons, I monitored 653 eggs from 113 nests. The maximum daily temperature throughout the egg formation period was 1.27 ºC ± 0.11 warmer at the wetland site than the agricultural site ($t = -11.04$, $df = 64$, $P < 0.001$), and 4.39 ºC ± 0.83 cooler in 2022 than 2023 ($t = -4.89$, $df = 128$, $P < 0.001$). Overall, 9.80% (64/653) of embryos failed to hatch. The odds of an embryo surviving to hatch increased as clutches were initiated later in the breeding season ($\beta = 0.37 \pm 0.17$, $P = 0.03$; Figure 3.2). Embryonic viability was not predicted by max temp T-2, female age, egg mass, or laying order, and did not differ between sites or years.

Warmer temperatures two days prior to egg laying were associated with heavier eggs ($\beta = 0.23 \pm 0.03$, $t = 7.10$, $P < 0.001$; Figure 3.3A), and egg mass increased with laying order ($\beta = 0.07 \pm 0.01$, $t = 5.98$, $P < 0.001$; Figure 3.3B). Mean egg mass was lower overall in 2022 (1.73 g ± 0.01) than in 2023 (1.75 g ± 0.01); however predicted egg mass in 2023 was lower than 2022 after controlling for the effects of temperature and laying order ($\beta = -0.21 \pm 0.08$, $t = -2.51$, $P = 0.01$; Table 3.1). Neither the minimum age of females nor site type predicted egg mass and both variables were removed from the final model during the backward selection process.

3.4.1 Tables

Table 3.1. Results of final generalized linear mixed models testing the effects of female quality and site conditions on prenatal reproductive success of female tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada. The z-value is reported for the binomial regression of embryonic viability, and the t-value is reported for the linear regression of egg mass. Results for year are reported for 2023 in comparison to 2022. Female identity was included as a random effect in all models, and 653 eggs were included in all analyses.

	β ± SE	t or z-value	P
Embryonic viability			
Clutch initiation date	0.37 ± 0.17	2.19	0.03
Egg mass			
Year	-0.21 ± 0.08	-2.51	0.01
Max temp T-2[1]	0.23 ± 0.03	7.10	< 0.001
Laying order	0.07 ± 0.01	5.98	< 0.001

[1] Maximum temperature two days before egg laying.

3.4.2 Figures

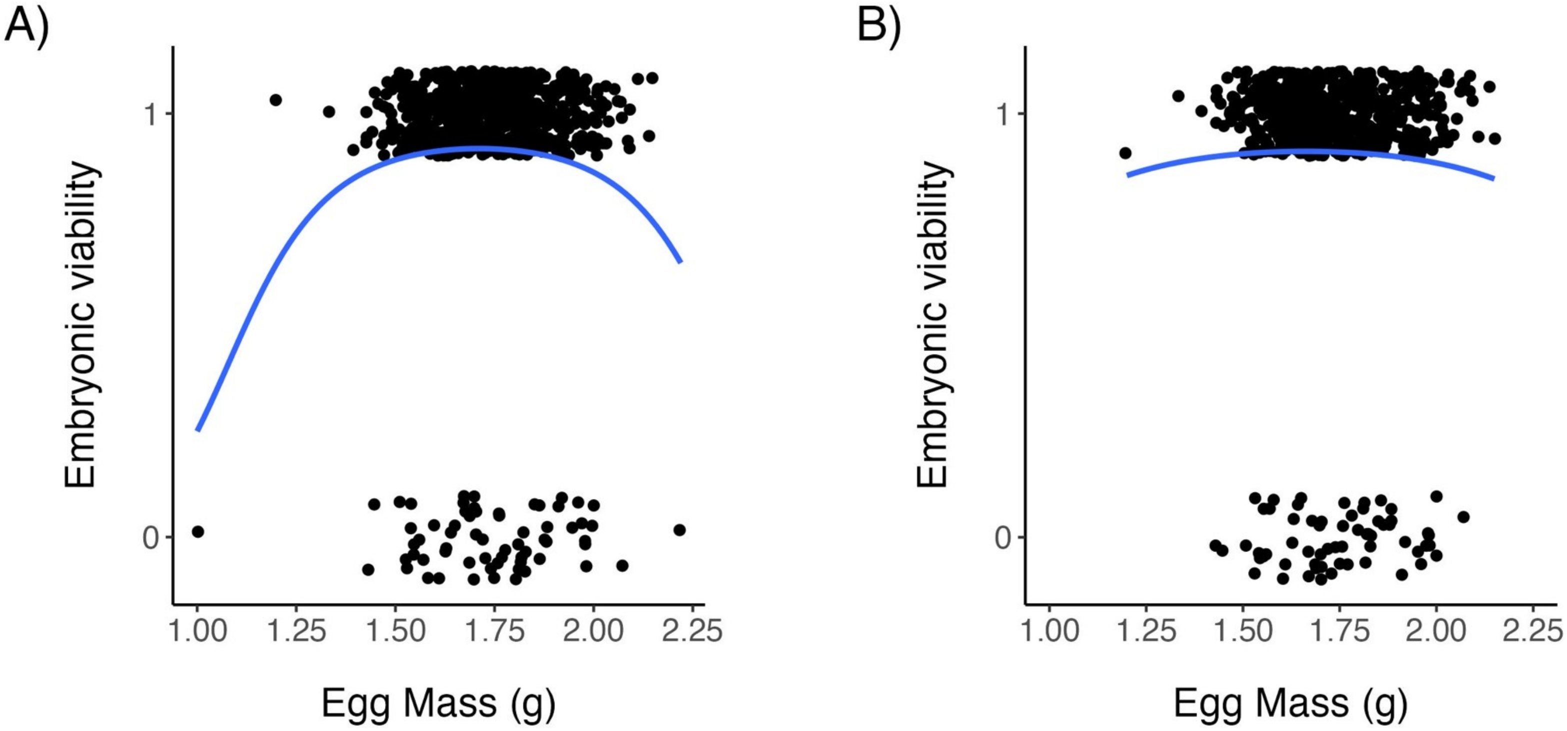


Figure 3.1. The quadratic relationship between viability (hatched = 1, failed to hatch = 0) and mass of eggs laid by tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada, in 2022 and 2023. The relationship is significant (estimate = -0.16 ± 0.08, z = -2.13, P = 0.03) when all eggs are included (A; n = 653), and non-significant (estimate = -0.07 ± 0.11, z = -0.67, P = 0.50) when the smallest egg (1.00 g) and largest egg (2.22 g) are removed from the dataset (B). Points are jittered along the y-axis to illustrate their binomial distributions.

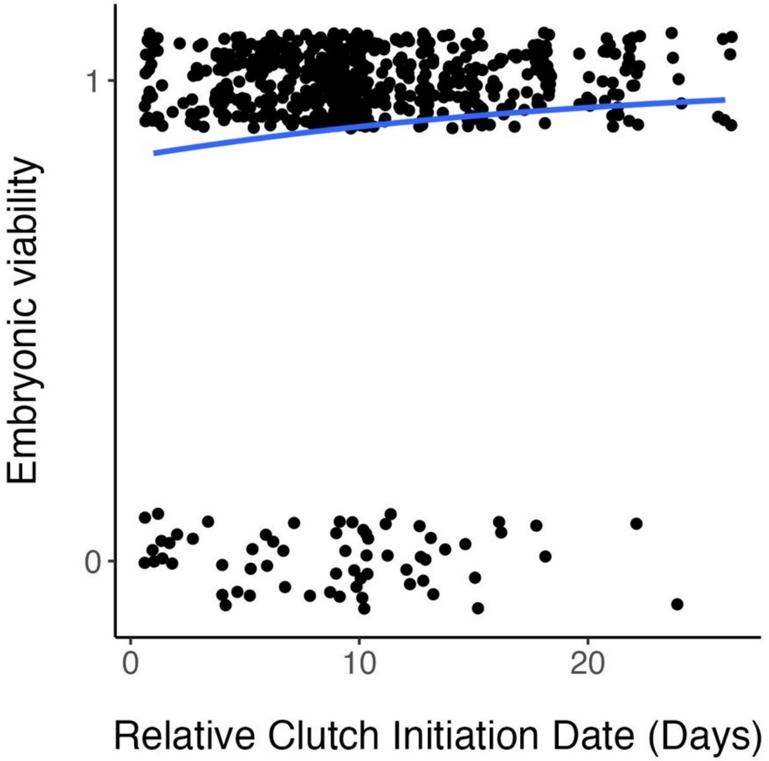


Figure 3.2. Embryonic viability in tree swallows (*Tachycineta bicolor*) breeding near Prince George, BC, Canada, in 2022 and 2023 increased as clutch initiation date increased ($\beta = 0.37 \pm 0.17$, $P = 0.03$; $n = 653$). Relative clutch initiation date is calculated as the difference between the date the first egg of the clutch was laid and one day prior to the date the first egg was laid at either site included in the study in each year (i.e., which had a relative hatch date of 1). Points are jittered along the y-axis to illustrate their binomial distributions (hatched = 1, unhatched = 0).

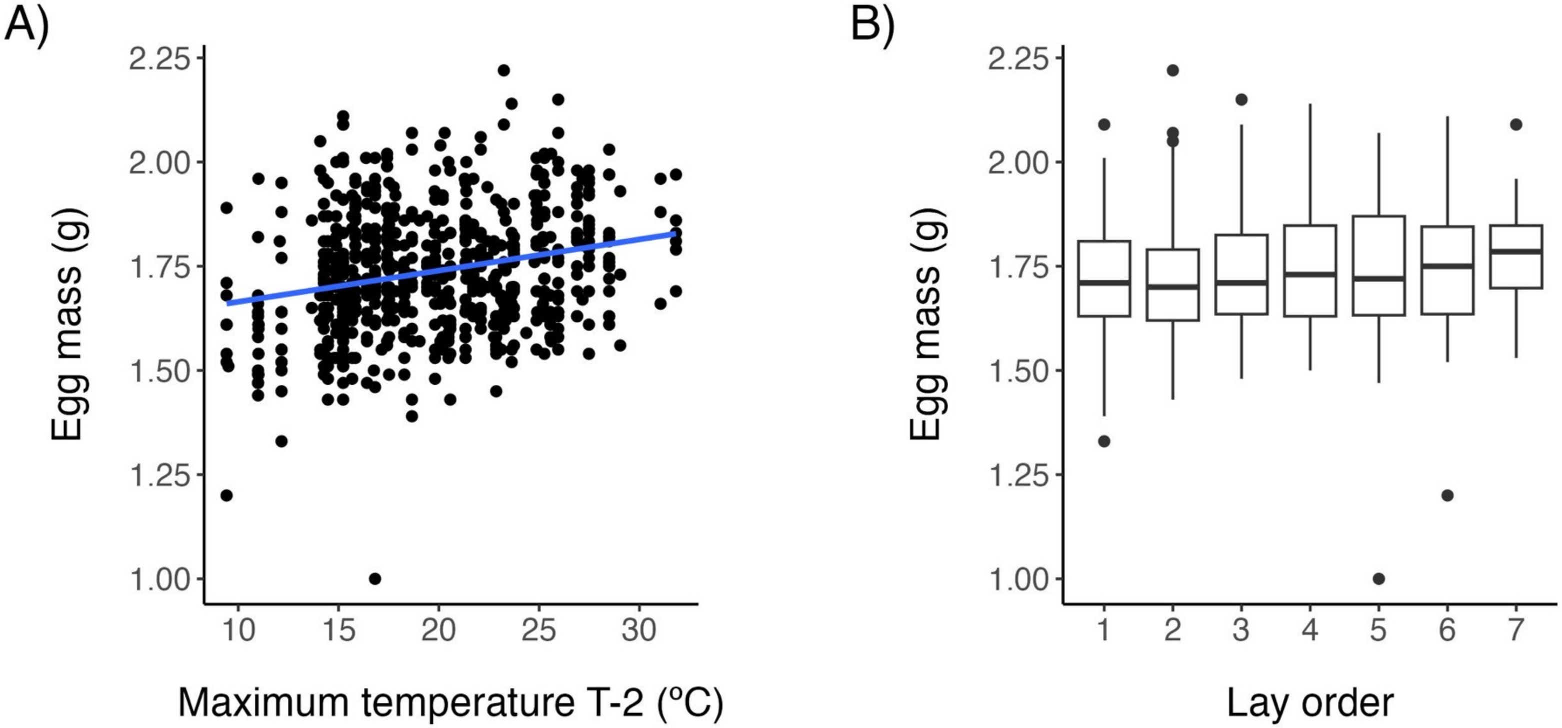


Figure 3.3. The mass of eggs (n = 653) laid by tree swallows (*Tachycineta bicolor*) breeding near Prince George, BC, Canada in 2022 and 2023 increased as maximum temperature two days prior to egg laying increased (A; $\beta = 0.23 \pm 0.03$, $t = 7.10$, $P < 0.001$) and as laying order increased (B; $\beta = 0.07 \pm 0.01$, $t = 5.98$, $P < 0.001$). Eggs were weighed one day after they were laid.

3.5 DISCUSSION

3.5.1 Embryonic viability

The causes of prenatal mortality in birds have been understudied, partially due to difficulties in differentiating between infertility and early embryonic mortality (Hemmings & Evans, 2020). My study provided an opportunity to examine factors contributing specifically to the survival of embryos because the fertility of the eggs monitored in this study was virtually 100% (Chapter 2). Contrary to expectations, embryonic viability increased as clutches were laid later in the breeding season. In tree swallows, the relative date a female lays her first egg is indicative of many measures of individual quality and reproductive success, including eggs with larger yolks, larger clutch sizes, and higher nestling survival (Ardia et al., 2006c; Dunn et al., 2011; Stutchbury & Robertson, 1988). Additionally, pairs with more combined breeding experience initiate egg laying earlier (Lombardo & Thorpe, 2010), which may be because they have site fidelity and are able to acquire nesting sites with more desirable microclimates (Ardia et al., 2006b). Embryos from higher quality, more successful females may therefore be expected to hatch more often, especially given that production of unhatched eggs represent a loss of maternal investment (Williams, 2005). Stutchbury & Robertson (1988) did not detect an association between hatching success and clutch initiation date of tree swallows breeding near Kingston, Ontario; however, they measured hatching success as a percentage per clutch (average number of viable eggs in a clutch/clutch size), rather than the viability rate of all individual eggs in the population, which may contribute to the difference in results. Importantly, tree swallows residing at the study sites near Kingston were nest site-limited and the population was composed of a group of breeding adults who were naturally successful at obtaining a nest site, and a group of floating adults who were provided additional nest sites later in the breeding season and included in the study. Conversely, nest box occupancy at the study sites near Prince George was not saturated; therefore, the natural settlement of breeders into nest sites and ultimately, the initiation of clutches, may be fundamentally different between the two geographic regions. Although female tree swallows who lay eggs earlier in the breeding season are qualitatively superior, embryonic development may be deleteriously affected by colder temperatures during this time period. This may occur as either a consequence of decreased egg content quality from a lower abundance of insect prey, or inferior incubation, as female tree swallows leave the nest more often in cold and dry conditions (Coe et al., 2015). Because earlier-breeding adults fledge more young (Dunn et al., 2011), there may be a trade-off between hatching success and juvenile survival in tree swallows, although Nooker et al. (2005) found that the number of eggs that hatched in a clutch increased fledging success.

Despite differences in breeding experience, embryonic viability was not affected by female age in this study. The delayed plumage maturation of SY female tree swallows presents an opportunity to accurately age females in their first breeding season, even when individuals are recruited from unbanded populations, and researchers often categorize females as SY or ASY to investigate the differences between them. Typically, ASY females are superior to their SY counterparts, as they have a higher body mass, initiate breeding earlier, lay larger eggs and clutch sizes, raise larger nestlings, and fledge more young (Ardia et al., 2006c; Beck et al., 2015; Bentz & Siefferman, 2013; Bitton & Dawson, 2017; Winker & Allen, 1996). While there are many measures of reproductive success in which SY females perform poorer than ASY females, it is possible that embryonic viability is not one of them; in fact, De Steven (1978) found that SY females had significantly higher hatching success in one year and non-significantly higher hatching success in the other year of her two-year study. Notably, I did not group females into SY and ASY categories, rather, I assigned a minimum age to each female based on a combination of plumage colour and banding information and used age as a continuous variable in my models. ASY females who did not hatch at either study site were therefore assigned a minimum age of two when they first bred at one of the study sites, so my results may be limited by the estimated ages of these females, although using continuous variables for demographic metrics such as age increases the statistical power of ecological regression models (Beltran & Tarwater, 2024).

3.5.2 Egg mass

Eggs are the first form of maternal investment birds allocate to their young, as the internal contents provide the resources necessary for the growth and hatching of embryos (Carey, 1996). Prenatal investment by female birds in their eggs has effects that can last through postnatal development and into adulthood (Williams, 2012). The total size of eggs is often considered a metric of prenatal maternal investment, and whole egg mass is used as a measure of reproductive success in many avian studies (e.g., Pellerin et al., 2016; Rotenberry & Balasubramaniam, 2020). Egg mass in tree swallows is strongly associated with individual female identity (Lombardo et al., 2021; Taff et al., 2022), but other maternal and environmental factors also influence the mass of eggs, both within and among clutches.

In this study, egg mass was positively associated with laying order, as has been observed in various other studies of the species (Ardia et al., 2006c; Bitton et al., 2006; Custer et al., 2010; Lombardo et al., 2021; Zach, 1982; but see Whittingham et al., 2007). The ultimate mechanism for increasing mass across laying order remains poorly understood, although research in many avian species has shown that females allocate different resources across the laying order to counteract intra-brood competition among nestlings (e.g., Hahn, 2011; Soma et al., 2007). Many female tree swallows initiate incubation after laying their penultimate egg, resulting in asynchronous hatching where the last-laid egg hatches 1 – 3 days after other eggs in the clutch begin to hatch (Zach, 1982). The prevalence of heavier eggs at the later end of the laying order in this species may therefore provide a temporary advantage to chicks hatching asynchronously from the last-laid eggs, since the mass of hatchlings is positively associated with egg mass (Bitton et al., 2006; Whittingham et al., 2007).

The maximum daily temperature two days prior to laying date was a significant predictor of egg mass, both in the final model and in the preliminary analysis of climate timing windows. This finding is consistent with a study of 585 tree swallow eggs that found that insect availability two to three days prior to egg laying predicted whole egg mass (Ardia et al., 2006c), as maximum daily temperature is strongly correlated with the insect prey of aerial insectivores (Garrett et al., 2022). Furthermore, higher ambient temperatures within one to three days prior to laying date have been associated with larger eggs in other studies of tree swallows (Pellerin et al., 2016; Whittingham et al., 2007). Lombardo et al. (2021) did not find an effect from maximum daily temperature, or any other environmental variable, averaged from one to five days prior to egg laying; however, this result may have been driven by the inclusion of the fourth and fifth days prior to egg laying, which are not associated with whole egg mass or yolk mass in tree swallows (Ardia et al., 2006c). Contrary to some studies (Ardia et al., 2006c; De Steven, 1978; Taff et al., 2022), female age did not predict egg mass; however, this result was consistent with Lombardo et al. (2021), who did not find an effect of age across multiple years, despite significant differences within two separate single-year periods. Despite overall warmer temperatures in 2023, egg mass was lower when compared to 2022 and may have been affected by a short cold snap and rainfall event that occurred during the egg laying period (see Chapter 4).

In a meta-analysis of 162 avian species, Krist (2011) determined that larger and heavier eggs were positively associated with hatching success, larger nestlings, higher growth rates and increased survival of juveniles. Four studies of tree swallows were included in the meta-analysis, and closer examination of these and more recent studies of egg mass yields less definitive associations between the size of eggs and reproductive success within the species. Heavier eggs produce larger hatchlings and four-day-old nestlings, but there is no influence of egg mass on the size of older nestlings or fledging success, and none of the studies of tree swallows included in the meta-analysis examined the relationship between egg mass and hatching success (Bitton et al., 2006; De Steven, 1978; Griebel & Dawson, 2019; Taff et al., 2022; Wardrop & Ydenberg, 2003; Whittingham et al., 2007). I did not find a significant effect of egg mass on embryonic viability in this study. Whereas a few studies of wild birds have detected a positive relationship between egg mass and hatching success (e.g., Perrins, 1996; Sanchez-Lafuente, 2004), various others have found no effect (e.g., Beissinger et al., 2005; Magrath, 1992; Vinuela, 1997), including a recent study of tree swallows and 13 other passerine species in Illinois (Di Giovanni et al., 2022). Lombardo et al. (2021) detected a positive trend between hatching success and egg mass in tree swallows, but only in eggs laid by SY females, and the effect size was small. In domesticated species, the relationship between egg mass and hatching success is quadratic, with the lightest and heaviest eggs failing to hatch at higher proportions than median-weighted eggs (Gonzalez et al., 1999; Kirikçi et al., 2010; Narushin & Romanov, 2002; Wilson, 1991). In this study, I noted that the lightest egg (1.00 g) and the heaviest egg (2.22 g) both failed to hatch and there was initially a significant quadratic relationship between egg mass and hatching success; however, when these two eggs were removed from the dataset, the relationship became non-significant. It is possible that a threshold for hatching on one or both ends of the egg mass spectrum for tree swallows exists, but a substantially larger dataset, particularly with more extra-small and extra-large eggs is required to explore this relationship further.

A complicating factor to consider when evaluating egg mass is that maternal investment is allocated differentially between the yolk, albumen, and shell components, and while the majority of essential nutrients and hormones are located in the yolk, albumen mass is closely associated with embryonic size and growth (Deeming, 2002). Larger eggs within some passerine species tend to contain relatively smaller yolks and higher albumen content (Remeš, 2011; Williams, 2012), and in some species, older females lay eggs with higher albumen content (Bogdanova et al., 2006). Ardia et al. (2006c) found that while older female tree swallows laid heavier eggs, there was no effect of age on yolk mass, indicating older female tree swallows may also lay eggs with a larger albumen to yolk ratio. Furthermore, the quality of insect prey, such as the abundance of omega-3 fatty acids, may be more important than the overall quantity consumed by tree swallows (Twining et al., 2016; Twining et al., 2018). The assumption that heavier eggs are of higher quality does not account for the composition and concentrations of key resources for embryos in yolks; therefore, a cautious approach should be used when considering the effects of maternal age on whole egg size as a measure of prenatal investment.

3.5.3 Feather colour

Preliminary analyses of feather colour demonstrated that female ornamentation of rump feathers did not predict prenatal reproductive success in this study. While this is the first study to examine the relationship between feather colour and embryonic viability or hatching success in tree swallows, two studies have previously tested the effect of ASY female rump colour on other metrics of reproductive success. In a study of 20 nests on a different study site near Prince George than the sites included in my study, Bitton et al. (2008) found that brighter females laid clutches with a higher mean egg mass and bluer females fledged more young. On a site containing 230 nest boxes in North Carolina, Bentz and Siefferman (2013) found that bluer and brighter females laid larger eggs but had lower immune responses and raised offspring in lower body condition; however, these results are not directly comparable to Bitton et al. (2008), as Bentz and Siefferman (2013) did not include UV chroma in the PCA of feather colour traits and therefore only tested one principal component combining brightness, blue chroma, and hue. Differences in results between my study and these studies could be due to statistical design, geographic area, or other co-varying factors of feather ornamentation as a signal of reproductive success that have yet to be explored. Additionally, while plumage characteristics may be important for other aspects of breeding, such as conspecific competition between females (Beck & Hopkins, 2019; Berzins & Dawson, 2016), assortative mating (Bitton et al., 2008), and extrapair fertilization success by males (Bitton et al., 2007; Whittingham & Dunn, 2016), these traits may not be important signals for embryonic viability.

3.5.4 Limitations and significance

There are other factors known to constrain embryonic viability in birds that were not included in this study. Early embryonic mortality, which accounted for the majority of prenatal mortality in this study population (Chapter 2), can be associated with genetic abnormalities (Shook et al., 1971) and by sperm counts lower than what is necessary for the physiological polyspermy required for normal avian embryo development (Hemmings & Birkhead, 2015). Developmental abnormalities can also cause mortality in later embryonic stages (e.g., Forstmeier & Ellegren, 2010), and one egg included in this study was found to contain conjoined twins (Appendix C). Later embryonic mortality can also be influenced by environmental conditions during incubation, such as temperature (Webb, 1987), and by parental behaviour, such as egg-turning (Deeming, 2009). Because female tree swallows are solo intermittent incubators, the frequency and duration of incubation off-bouts may likewise affect the ability of embryos to hatch. A dataset containing a larger number of eggs with embryos that died in middle and late stages is required to fully evaluate the effects of environmental conditions and maternal investment during the incubation period. Conversely, significant associations between factors can vary yearly in tree swallows (Lombardo et al., 2021), so my study provides a starting point to which future studies with larger data sets and longer time periods can build upon. Additionally, this study is the first to my knowledge to examine factors that specifically constrain embryonic viability in tree swallows, rather than overall hatching success, and provides a baseline for gaining insights into prenatal reproductive success of an aerial insectivore.

Chapter 4: Responses of female tree swallows to a cold snap during the egg laying period

4.1 ABSTRACT

Wildlife species that breed annually in specific seasons rely on cyclical changes to ambient conditions that determine food abundance and ultimately the energy balance available for successful reproduction. Increased variability in local weather from climate change poses a threat to individuals unable to respond effectively to sudden changes in environmental conditions. The effects of climate change on the nestling period in birds have been well documented; however, less is understood about the plasticity of prenatal maternal investment in response to variability in local environmental conditions. In species where females lay one egg every day from clutch initiation to clutch completion, laying skips can occur where at least one day passes without an egg being laid between clutch initiation and completion. Because many birds wait to start incubation until their clutches are complete or nearly complete, laying skips extend the onset of incubation for eggs laid earlier in the clutch. Hatchability may decrease when the period that eggs remain in the nest without incubation increases; therefore, individuals that delay incubation, through laying skips or behaviourally delaying the onset of incubation, may experience higher hatching failure of earlier-laid eggs. Tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada, in 2023 experienced a sudden and short cold snap during the egg laying period after multiple days of warm and dry conditions, and this event provided a unique opportunity to assess the effects of a short but harsh period of environmental conditions on prenatal maternal investment and behaviour. I investigated the effects of the cold snap on 101 females and their clutches across three study sites in the region. Most females did not initiate incubation during the cold snap and nearly half of the females laying during the cold snap skipped at least one day of egg laying. Individual quality did not predict whether a female would have a laying skip; however, females with laying skips laid heavier eggs, and females with larger clutches were more likely to have at least one laying skip. Laying skips were not directly related to embryonic viability (i.e., an embryo surviving to hatching); however, laying skips and delayed incubation collectively increased the number of days between laying and the onset of incubation of earlier-laid eggs, which may have decreased hatching success. This case study provides insight into how birds dependent on short-term availability of resources, such as aerial insectivores, respond to sudden fluctuations in environmental conditions during the prenatal investment period.

4.2 INTRODUCTION

Wildlife species that breed annually in specific seasons rely on cyclical changes in ambient conditions that determine food abundance and ultimately the energy balance available for successful reproduction (Bronson, 2009; Carey, 2009; Somveille et al., 2018). When these regular cycles are disrupted and local conditions abruptly and adversely change during the breeding season, individuals must react quickly to maximize fitness. As climate change continues at an unprecedented rate (Maibach et al., 2014), increased variability in local weather poses a threat to individuals unable to respond effectively to sudden changes in daily temperature and precipitation. Recent shifts in climate regimes are contributing to steep population declines, due in part to decreased foraging efficiency and habitat availability (reviewed in Jenouvrier, 2013). Additionally, warming temperatures are leading to advanced timing of breeding, yielding potential mismatches between the nestling period and peak food abundance in birds (e.g., Dunn, 2019). Increased variability in weather patterns and the occurrence of extreme weather events adds a complicating factor to the advancement of the breeding period, yet less is understood about the plasticity of prenatal maternal investment in response to variability in local environmental conditions.

Avian egg formation is initiated prior to egg laying in a period called rapid yolk deposition (RYD), in which yolk precursors are synthesized in the liver and deposited in follicles in the ovary of the female as proteins and lipids, which constitute a mature yolk (Williams, 2012). In birds that normally lay one egg per day, follicles are activated sequentially and initiate RYD one day apart (Williams, 2012), with the rate of deposition reaching a maximum in the last half of the RYD period (Ojanen, 1983). Follicles of birds that lay one egg per day are commonly labelled F1 through F4 or higher, with F1 referring to the most matured follicle ready for ovulation and the highest F-number referring to the least matured follicle at a given time. The number of activated follicles depends on the length of time required for RYD, which is species-specific. After RYD, the follicle is ovulated, and the remainder of an individual egg is formed in the reproductive tract, ending in oviposition when the female evacuates the egg through her cloaca (Williams, 2012). At this stage, embryonic development is paused if the female does not immediately initiate incubation. The minimum temperature required for embryo development, referred to as "physiological zero", is approximately 24 – 27 ^{o}C and embryos kept below this temperature enter a suspended state (Wilson, 1990).

Generally, most passerines lay one egg every day from clutch initiation to clutch completion; however, laying skips (also referred to as "laying gaps") can occur where at least one day passes without an egg being laid between clutch initiation and completion. Two mechanisms have been identified as possible proximate causes for laying skips. Domestic poultry researchers commonly refer to the period when ovulation can occur as the “open period”, which is thought to be an 8- to 10-hour window every morning when luteinizing hormone is released (Johnston & Gous, 2003). If the F1 follicle matures after this window, laying is skipped for the day and the female will ovulate the F1 follicle the following morning in her next open period. Because domestic chickens (*Gallus gallus domesticus*) are indeterminate layers and typically lay one egg every 25 hours, hens will naturally skip a day later in their laying cycles when the timing of egg formation inevitably becomes mismatched to their open periods, which are on a 24-hour cycle (Williams, 2012). The open period mechanism does not adequately explain laying skips in wild passerines, particularly when females experience laying skips early in their clutches or for multiple days at a time. Follicular atresia has been attributed to these cases, in which one or more follicles cease developing and are reabsorbed prior to ovulation (Guraya, 1989). Follicular atresia has been linked to sex-specific selective abortion (reviewed in Alonso-Alvarez, 2006), and periods of stress including unseasonably low ambient temperatures and decreased food availability (e.g., Eeva & Lehikoinen, 2010; Nilsson & Svensson, 1993).

Because many birds wait to initiate incubation until their clutches are complete or nearly complete (Clark & Wilson, 1985), laying skips extend the lay-to-set intervals (the number of days between when an egg is laid and when incubation begins) for the earlier-laid eggs in the clutch. Hatchability may decrease when the period that eggs remain in the nest without incubation increases (Aldredge, 2017; Beissinger et al., 2005); therefore, individuals that delay incubation may experience higher hatching failure of earlier-laid eggs (e.g., Arnold et al., 1987; Sockman, 2008), and this effect may be exacerbated if individuals have laying skips or further delay the onset of incubation past laying of the last egg in the clutch.

Decreases in hatching success linked to long lay-to-set intervals have been attributed to prolonged exposure of eggs to suboptimal ambient temperatures. In cases where an egg remains unincubated in warmer temperatures between physiological zero and optimal incubation temperature of the species, embryonic development may continue abnormally (Deeming & Ferguson, 1992; Webb 1987), or warm and moist conditions may promote fungal or bacterial infection of the egg (Cook et al., 2005; Shawkey et al., 2009). If an egg is exposed to colder temperatures for extended time periods, viability may also decrease, although the mechanism for embryonic mortality in these cases is less understood (Fasenko, 2007). Conversely, delaying hatch may be beneficial to realign the nestling period with peak food abundance in species that advance the timing of breeding (Both & Visser, 2005; Cresswell & McCleery, 2003; Tomás, 2015), or may be necessary for breeding adults to replenish sufficient body fat reserves to provide adequate care to embryos during incubation, as incubation can be energetically costly (Nord & Williams, 2015). Little is known about the measures of female quality that may predict the occurrence of laying skips during periods of environmental stress, and whether delayed hatching is ultimately beneficial or detrimental for reproductive success.

The tree swallow (*Tachycineta bicolor*) is an aerial insectivorous species that has advanced egg laying dates in populations across its breeding range over the past half-century (Bourret et al., 2015; Dunn et al., 2011; Shipley et al., 2020). Tree swallows breeding near Prince George, British Columbia, Canada, in 2023 experienced a sudden cold snap during the egg laying period after multiple days of warm and dry conditions. The cold snap was characterized by a rapid decrease in ambient temperature and a period of precipitation over two days, followed by more warm and dry weather. This event provided a unique opportunity to assess the effects of a short but harsh period of environmental conditions on prenatal maternal investment and behaviour. Whereas researchers have reported effects of similar cold and wet weather events on nestling growth, survival, and immune function of tree swallows (e.g., Garrett et al., 2022; Griebel & Dawson, 2019; Winkler et al., 2013), effects on embryonic viability (i.e., an embryo surviving to hatching) and plasticity of egg laying and incubation behaviour are mostly unknown.

I predicted that females would have had difficulty obtaining sufficient energy required for prenatal parental care during the cold snap, so females due to begin incubation during the cold snap would delay the onset of incubation. Similarly, I expected that females who had laying skips immediately following the cold snap would have experienced follicular atresia due to their inability to forage effectively and would therefore be of lower quality than females who did not have laying skips. I used measures of the combined length of the head and bill, length of wing, body condition, age, clutch initiation date, clutch size, and plumage reflectance as metrics of female quality, as these variables have been associated with female quality in previous research on tree swallows (e.g., Beck et al., 2015; Bitton et al., 2008; Dunn et al., 2011; Pellerin et al., 2015; Winkler & Allen, 1996). Because laying skips provide females with more time to provision their eggs, I also predicted that eggs laid by females with laying skips after the cold snap would be heavier than those laid by females without laying skips. Finally, I predicted that eggs with longer lay-to-set intervals would have lower embryonic viability, and therefore, females with laying skips would be more likely to lay eggs with lower hatching success.

4.3 MATERIALS AND METHODS

4.3.1 Study area, study species, and general field methods

Tree swallows are migratory aerial insectivores and secondary cavity nesters that breed across Canada and the northern United States in open, semi-treed habitat usually near water (Winkler et al., 2020a). Their diet is composed of small flying insects caught in flight, such as dipterans, hemipterans, and coleopterans (Harris, 2023). Tree swallows are “income breeders”, rapidly using nutrients to form eggs shortly after consuming food (Jönsson, 1997), and insect abundance during the egg formation period has been positively associated with larger clutches, heavier eggs, and shorter incubation periods (Hussell & Quinney, 1985; Nooker et al., 2005). The RYD period of tree swallows is five to six days and yolk mass is most influenced by insect abundance two to three days before an egg is laid (Ardia et al., 2006c); therefore, eggs are likely heavier and contain superior nutrition for embryos when insect abundance is higher during this stage of the RYD period. Additionally, the abundance of flying insects is directly associated with local weather conditions at the breeding sites of insectivorous birds (Cox et al., 2019; Grüebler et al., 2008), so eggs laid immediately following warmer and drier site conditions are likely to be heavier and may contain higher quality yolks, resulting in higher hatching success. Female tree swallows lay one egg per day with clutch size usually ranging from 4 – 7 eggs and begin a 13 to 14-day incubation period after either the penultimate or final egg is laid (Zach, 1982). Females are solo intermittent incubators, providing the entirety of care to their eggs during incubation and leaving their nests multiple times per day for foraging bouts (Winkler et al., 2020a).

Common measures of female quality in tree swallows include feather colour and female age. Adult tree swallows have dorsal plumage with a blue-green iridescence produced by the interaction between light waves and the nanostructure of the feathers (Prum, 2006). While males grow adult plumage following their first post-fledging molt, second-year (SY) females (who are in their first year of breeding) have delayed plumage maturation and display a high proportion of brown dorsal feathers before developing adult plumage as after-second-year (ASY) females (Hussell, 1983). This retention of juvenile colouring is thought to be a strategy to signal inexperience and reduce conspecific aggression from ASY females (Beck & Hopkins, 2019; Coady & Dawson, 2013). In addition to inexperience in breeding, SY females generally have lower reproductive success than ASY females (Ardia et al., 2006c; Beck et al., 2015; Bentz & Siefferman, 2013; Bitton & Dawson, 2017; Winker & Allen, 1996). Within the ASY age class, females with brighter and bluer feathers produce heavier eggs (Bitton et al., 2008; Bentz & Siefferman, 2013).

I studied tree swallows breeding in nest boxes near Prince George, British Columbia, Canada (53° N, 123° W), in 2023 at three established study sites – an agricultural site, a wetland site, and a mixed habitat site (composed of farmland with a small pond adjacent to the nest boxes). This area in central British Columbia is in the Sub-Boreal Spruce biogeoclimatic zone, which is characterized by annual fluctuations in temperature extremes between summer and winter, and moderate precipitation (Meidinger et al., 1991). Nest boxes at all sites were mounted ~1.5 m above ground approximately 30 m apart on fence posts in hay fields, grazing pastures, or near wetlands. There were 131, 60, and 18 boxes available for use at the agricultural, wetland, and mixed habitat sites, respectively. Local temperature (°C) and precipitation (mm) data were collected at the agricultural and wetland study sites with weather stations (Onset Computer Corp.) in one-minute intervals throughout the breeding season. In lieu of site-specific weather data at the mixed habitat site, I used historical temperature data collected at the Prince George airport, located approximately 16 km from the study site (Government of Canada, 2025). Precipitation data are not publicly available for this time period at the Prince George airport.

Nest boxes were emptied of all nesting material at the end of each breeding season. I began checking nest boxes every two days beginning in early May to monitor nest construction, and then daily throughout the nest building and egg laying stages from mid-May to mid-June. Eggs were sequentially numbered using a non-toxic pen on the day they were laid and weighed with a digital scale (nearest 0.01 g) one day after they were laid. A clutch was considered complete when incubation had started and three consecutive days had passed without a new egg being laid.

Once hatching was complete, I trapped females (n = 101) in their nest boxes as they entered to feed nestlings. I measured each female, including body mass (using a spring scale, nearest 0.25 g), length of the wing chord (using a ruler; nearest 0.5 mm), and the combined length of the head and bill (using calipers; nearest 0.01 mm). The minimum age of each female was determined based on plumage colouration and previous banding information. Four females, who collectively laid 25 eggs, known to be a minimum of five or six years old were conservatively assigned a minimum age of four to decrease the risk of an outlier effect from the older ages. Body condition was calculated by deriving the residuals from a regression between body mass and length of the head-bill, with the number of days following hatch that the measurements were obtained as a covariate from all females included in the study.

4.3.2 Feather colour analysis

During banding and measuring of each female, I collected a minimum of four rump feathers for spectral analysis and stored each set of feathers in a manila envelope at room temperature. Because SY females have delayed plumage maturation, I only included ASY females in feather colour analyses. To process the feathers, I arranged four feathers so that the shafts were taped to a piece of black cardboard and the feathers partially overlapped each other to mimic the natural arrangement of rump feathers on an individual tree swallow (Bitton et al., 2007). I placed each sample on a black background and measured colour spectra using a USB2000 spectrometer and a deuterium-halogen light source (Avantes, Apeldoorn, The Netherlands). Using a bifurcated probe in a black holder that excluded ambient light and held at 90º to the surface of the feathers, I took three measurements per sample, resetting the probe between measurements. I recorded spectral reflectance data using OOIBase32 software (Ocean Optics, Orlando, Florida) and calculated reflectance following Berzins & Dawson (2016).

I completed spectral analysis using the "pavo" package (Maia et al., 2019) in R version 4.3.1 (R Development Core Team, 2013). I smoothed the spectral curves and quantified the mean of each set of three measurements for four reflectance characteristics that are demonstrated to indicate the overall quality of ASY female tree swallows (e.g., Bitton et al., 2008): brightness (mean reflectance between 300 – 700 nm), hue (the wavelength of maximum reflectance), ultraviolet (UV) chroma (the proportion of light reflected from 300 – 400 nm relative to the entire spectrum), and blue chroma (the proportion of light reflected from 400 – 512 nm relative to the entire spectrum). I completed a principal component analysis to combine the four spectral characteristics. The first principal component explained 63% of the total variation and was weighted by greater UV chroma and blue chroma; therefore, higher scores were associated with colour reflected at shorter wavelengths and were bluer (factor loadings: brightness = 0.20, UV chroma = 0.55, blue chroma = 0.56, hue = -0.59). The second principal component explained 30% of the total variation and was weighted by brightness (factor loadings: brightness = 0.84, UV chroma = 0.32, blue chroma = -0.31, hue = 0.30).

4.3.3 Incubation initiation and lay-to-set interval determination

Once egg laying commenced, I placed a temperature datalogger (Thermocron iButton DS1921G, Maxim Integrated, San Jose California; accuracy ±0.5 °C) in the nest cup slightly above the eggs to record the temperature of the nest, and another datalogger in an upper corner of the box to record ambient temperature. Dataloggers were set to record temperature at 20-minute intervals. The loggers were collected from the nest boxes after eggs hatched and temperature data were downloaded. As temperature data were not available until each datalogger was retrieved from a nest, I also estimated the date each female started incubation by assessing the surface temperature of the eggs using a digital infrared thermometer (nearest 0.1 ºC). I noted whether the female flushed from the nest box upon approach, as the surface temperature of eggs will cool when females leave the nest to forage (Ardia et al., 2009). Generally, the surface temperature of eggs that were being incubated was at least 28 ºC, but unattended eggs that were being incubated were slightly cooler than eggs incubated by a female that flushed as I arrived at the box. I subsequently used a combination of data from the temperature dataloggers and infrared thermometer to determine the date when each female initiated incubation. Additionally, I cross-referenced the estimated date that initiation commenced using photographs taken when eggs were candled three to six days into incubation to determine developmental stages of embryos (see Chapter 2; Hemmings &

Birkhead, 2016; Murray et al., 2013). I calculated the lay-to-set interval for each egg by subtracting the ordinal lay date from the ordinal incubation initiation date.

4.3.4 Determination of the critical period of rapid yolk deposition (RYD)

All statistical analyses were conducted in R version 4.3.1 (R Development Core Team, 2023). I used the "climwin" package (Bailey & van de Pol, 2016; van de Pol et al., 2016) to assess the relationship between temperature during the egg formation period and the mass of eggs laid by tree swallows during my study period. The "climwin" package uses Akaike Information Criterion scores corrected for small sample sizes (AIC_c) to compare the fit of regressions between a biological response variable and an environmental predictor variable over different time windows. If one or more regressions fit better than a null model, the package will assist in identifying time periods where the biological response variable is most sensitive to the environmental predictor variable. Within the "slidingwin" function in this package, I used generalized linear mixed effects models fit with the "lme4" package (Bates et al., 2015) and included female identity as a random effect. I tested the quadratic relationship, fit using a quadratic term in a linear model, between maximum daily temperature and egg mass for all possible daily time windows within 1 – 7 days before each egg was laid, as maximum daily temperature is quadratically related to the abundance of insect prey for tree swallows (Garrett et al., 2022). The most parsimonious model had maximum daily temperature that both started and ended two days before egg lay (AIC_c weight = 0.99); therefore, the maximum daily temperature two days prior to the date each egg was laid (max temp T-2) was used in analyses.

4.3.5 Effects of the cold snap

To assess the effects of the cold snap that occurred 22 – 23 May 2023 on egg laying patterns and the onset of incubation, I divided breeding females into three groups according to whether the cold snap could have affected their RYD and/or egg laying: 1) Pre-Cold: Females who completed their clutches before or during the cold snap and so RYD would have been unaffected by it (Females W-3 to W-16, Figure 4.1); 2) Cold: Females who were undergoing the critical period of RYD of at least one egg during the cold snap (Figure 4.2); 3) Post-Cold: Females who initiated their clutches two or more days after the cold snap (i.e., no eggs were undergoing the critical period of RYD during the cold snap; Figure 4.3). One female who completed her clutch on 24 May was included in Group 1 because she laid her first egg on 15 May and had 5 days of laying skips preceding, and therefore unrelated to, the cold snap.

I quantified instances of delayed incubation, laying skips, and clutch abandonment within all three groups, and abandoned clutches were subsequently excluded from further analyses. I assumed that laying skips that occurred following the cold snap within the Cold group were likely in response to the cold snap, whereas laying skips before the cold snap (i.e., the Pre-Cold group) or substantially after the cold snap (i.e., the Post-Cold group) were due to other factors. I therefore evaluated factors that may predict the occurrence and number of laying skips in response to the cold snap exclusively using females in the Cold group. In this study, one laying skip = 1 day without egg laying between clutch initiation and completion, so females with multiple laying skips did not lay for multiple days and laying skips could be sequential or split throughout the laying period.

To investigate how the cold snap (i.e., ambient temperature) and laying skips may have affected egg mass, I tested if these factors influenced the mass of eggs laid immediately after the cold snap (i.e., laid on 24 May – 01 Jun) by females who were undergoing the critical period of RYD during the cold snap (Cold group). Additionally, I assessed the embryonic viability of all eggs that may have been affected by the cold snap in any way (i.e., eggs that were incubated during the cold snap, eggs that had delayed incubation due to the onset of incubation by females or laying skips, or eggs with a critical period of RYD during the cold snap). Abandoned, depredated, and broken eggs were removed from embryonic viability analyses (n = 77). Additionally, a portion of nests at the agricultural site were experimentally altered following incubation initiation for a separate study, and eggs from these clutches (n = 107) were also excluded from analyses of embryonic viability. I collected and assessed the fertility status of all remaining unhatched eggs and determined no eggs included in my study were infertile (Chapter 2).

4.3.6 Statistical analyses

For all other analyses, I used generalized linear models and generalized linear mixed effects models fit using the "lme4" package (Bates et al., 2015), with P-values generated by the "lmerTest" package (Kuznetsova et al., 2017). Females in the Cold group were assigned a binomial status if they had at least one laying skip (1) or did not have a laying skip (0), and eggs were assigned a viability status of hatched (1) or failed to hatch (0). Binomial logistic regressions with a logit link function were used when either of these variables was tested as the response variable. Significance was set at alpha = 0.05. Regression coefficients (β) and means are reported with ± 1 standard error. I used a backward stepwise elimination procedure to remove variables that did not approach significance ($P > 0.10$), and I used the "DHARMa" package (Hartig, 2024) on the saturated models to ensure models passed dispersion and outlier tests for binomial logistic regressions and normality, dispersion, and outlier tests for linear regressions. Additionally, I determined generalized variance inflation factor values for all saturated models were < 4 (i.e., low collinearity among predictor variables) using the "car" package (Fox & Weisberg, 2019). Saturated model sets are summarized in Appendix D.

To determine if laying skips in response to the cold snap were predicted by measures of female quality, I assessed the effects of wing chord length, body condition, age, clutch initiation date, clutch size, and feather reflectance PC1 and PC2 values on the occurrence of laying skips within the Cold group. Because feather reflectance values were not collected for SY females due to their delayed plumage maturation, two model sets were used for this analysis: one with females of all ages and feather reflectance variables excluded, and one without SY females and feather reflectance variables included. Using a subset of females in the Cold group that had at least one laying skip (n = 23), I then tested if the same factors without feather reflectance variables predicted the number of laying skips. Removing the SY females and adding feather reflectance variables to this subset created collinearity issues, so I did not use a second model set to test if feather reflectance was related to the number of laying skips. Study site was included as a covariate in these models.

I tested the effects of clutch initiation date, female age, max temp T-2, laying order, and whether the egg was laid by a female with at least one laying skip, on the mass of eggs laid by the Cold group after the cold snap (24 May and onward). I next assessed clutch initiation date, individual laying date of each egg, female age, max temp T-2, and lay-to-set interval on the embryonic viability of all eggs laid or formed before or during the cold snap. This dataset included all eggs laid on 26 May or earlier by females in the Pre-Cold and Cold groups. Eggs in the Pre-Cold group were being incubated or awaiting the onset of incubation, whereas those in the Cold group were awaiting the onset of incubation or undergoing RYD during the cold snap until 24 May as egg mass was most closely associated with maximum temperature two days prior to laying. To investigate if females in the Cold group that responded to the cold snap with laying skips laid eggs less likely to hatch, I then assessed these same predictors on the embryonic viability of eggs laid only by these females and added an additional variable representing whether the egg was laid by a female with at least one laying skip. In all models assessing egg mass and viability, study site was included as covariate and female identity was included as a random effect.

4.4 RESULTS

In 2023, I monitored 51, 35, and 15 nests at the agricultural, wetland, and mixed habitat sites, respectively. Egg laying began in mid-May when maximum daily temperatures were higher than normal for the time of year (25 – 31 ºC) and there was no precipitation. Data available from the Prince George airport for 2010 – 2022 shows that the mean maximum daily temperature for the last half of May (15 – 31 May) in this time period was 18.5 ºC (Government of Canada, 2025). From 17:00 hr on 21 May to 08:00 hr on 24 May (63 hours), ambient temperature decreased to a range of 6 – 13 ºC at all study sites. Totals of 7.0 mm and 11.4 mm of precipitation were recorded at the agricultural and wetland sites, respectively, from 17:00 hr on 21 May to 02:00 hr on 24 May (57 hours). Following the cold snap, weather conditions were warm and dry, and comparable to those before the cold snap (Figure 4.4).

4.4.1 Responses by females to the cold snap

The Pre-Cold group included 14 breeding females expected to initiate incubation during the cold snap on 22 or 23 May, and 13 of these females (92.86%) delayed incubation until 24 or

25 May (Figure 4.1). One of these females began incubating on 21 May, did not incubate on 22 or 23 May, and began incubating again on 24 May. There were no instances of clutch abandonment within the Pre-Cold group, and two females within this group (14.29%) had laying skips preceding the cold snap.

Among females who were undergoing the critical period of RYD of at least one egg during the cold snap (Cold group; n = 58), there were six instances of clutch abandonment before the onset of incubation. Of the remaining 52 females in the Cold group, 23 (44.23%) had at least one laying skip, and laying skips ranged from 1 – 5 days. Of the females who had at least one laying skip, the first skip usually occurred after one of the first three eggs was laid (73.91%; 17/23) and within the 23 – 25 May time period (95.65%; 22/23). None of the 9 females that laid their penultimate egg on 23 May began incubating until 24 or 25 May (Figure 4.2).

Among females who initiated their clutches two or more days after the cold snap (Post-Cold group; n = 25), no females initiated their clutches on 25 or 26 May, and all began laying on 27 May. There were two instances of clutch abandonment in the Post-Cold group. Of the 23 remaining clutches, 2 (8.70%) were laid by females who had laying skips, and 1 (4.35%) was laid by a female who delayed incubation (Figure 4.3). All other 20 (86.96%) clutches were laid by females who did not have laying skips and who initiated incubation after laying their penultimate or final eggs.

When females of all ages in the Cold group were included in analyses of predictors of laying skips, the odds of a laying skip occurring following the cold snap increased as clutch size increased ($\beta = 0.99 \pm 0.50$, $z = 1.99$ $P = 0.05$). The odds of a laying skip were also higher at the mixed study site ($\beta = 1.87 \pm 0.83$, $z = 2.27$, $P = 0.02$) and the wetland study site ($\beta = 1.51 \pm 0.89$, $z = 1.69$, $P = 0.09$) than at the agricultural study site, respectively. These trends all persisted when SY females (n = 12) were removed from the dataset (Table 4.1), and no other variables tested predicted the occurrence of laying skips.

After removing all females who did not respond to the cold snap with a laying skip, there were 21 females in the Cold group that had a laying skip of at least one day (range 1 – 5) and who were trapped and measured (two females had their clutches depredated and subsequently abandoned their nests before trapping occurred). Within this subset of females, the number of laying skips decreased with wing chord length (β = -0.88 ± 0.27, t = -3.21, P = 0.01; Figure 4.5A) and increased with age (β = 0.61 ± 0.30, t = 2.05, P = 0.06; Figure 4.5B), although the latter trend was not significant. Using a linear regression to test if wing chord increased with age, I found that age and wing chord were not significantly related, after controlling for study site (β = 0.12 ± 0.14, t = 0.90, P = 0.37). Females at the agricultural study site experienced fewer laying skip days (1.91 ± 0.42) than females at the mixed habitat study site (2.86 ± 0.55; β = 1.33 ± 0.62, z = 2.13, P = 0.05) and wetland study site (3.60 ± 0.75; β = 1.57 ± 0.77, z = 2.03, P = 0.06), respectively. When the wetland site was used as the reference level in these model sets, there was no statistically significant difference between the wetland and mixed habitat study sites in terms of the occurrence of at least one laying skip, nor the number of laying skips. No other factors tested (i.e., clutch initiation date, head-bill length, or body condition) predicted the occurrence or number of laying skips within the Cold group.

4.4.2 Egg quality

The mass of eggs laid by the Cold group after the cold snap were heavier when temperatures two days prior to laying were warmer (β = 0.24 ± 0.06, t = 3.73, P < 0.001; Figure 4.6A). Eggs were heavier when laid by females with laying skips (1.80 g ± 0.02) than females without laying skips (1.62 g ± 0.01; β =0.78 ± 0.37, t = 2.11, P = 0.04; Figure 4.6B). There was also a trend for egg mass to increase with laying order, but the relationship only approached significance (β = 0.08 ± 0.05, t = 1.81, P = 0.07; Table 4.2). There were 192 eggs included in this study that were laid or formed before or during the cold snap, and 12.5% (24/192) failed to hatch. The odds of an embryo being viable in these eggs increased with later clutch initiation dates (β = 0.77 ± 0.37, z = 2.07, P = 0.04; Table 4.2). Of the eggs laid by females in the Cold group, 7.53% (14/186) failed to hatch and lay-to-set intervals ranged 0 – 10 days (2.84 ± 0.19). Eggs in this group with longer lay-to-set intervals had lower viability, although the relationship was not statistically significant (β = -0.18 ± 0.10, z = -1.86, P = 0.06; Table 4.2). No other factors tested in analyses of embryonic viability approached significance.

4.4.3 Tables

Table 4.1. Results of final generalized linear mixed models testing the effects of female quality on the occurrence and number of laying skips of female tree swallows breeding near Prince George, British Columbia, Canada, following a cold snap in the egg laying period of 2023. The occurrence of at least one laying skip was tested twice: once to include females of all ages and once to only include after-second-year (ASY) females and their associated plumage reflectance. The z-value is reported for the binomial regressions of occurrence of at least one laying skip, and the t-value is reported for the linear regression of number of laying skips of females that had at least one skip. The agricultural site was set as the reference level for study site, and wing chord length was scaled to the z-distribution. Bolded P-values are considered significant at alpha = 0.05.

	β ± SE	t or z-value	P
Occurrence of at least one laying skip – all ages (n = 52)			
Clutch size	0.99 ± 0.50	1.99	**0.05**
Study site			
Mixed habitat	1.87 ± 0.83	2.27	**0.02**
Wetland	1.51 ± 0.89	1.69	0.09
Occurrence of at least one laying skip – ASY females (n = 40)			
Clutch size	1.26 ± 0.65	1.93	**0.05**
Study site			
Mixed habitat	2.31 ± 0.97	2.39	**0.02**
Wetland	1.96 ± 1.05	1.87	0.06
Number of laying skips – all ages (n = 21)			
Minimum age (years)	0.61 ± 0.30	2.05	0.06
Wing chord length (mm)	-0.88 ± 0.27	-3.21	**0.01**
Study site			
Mixed habitat	1.33 ± 0.62	2.13	**0.05**
Wetland	1.57 ± 0.77	2.03	0.06

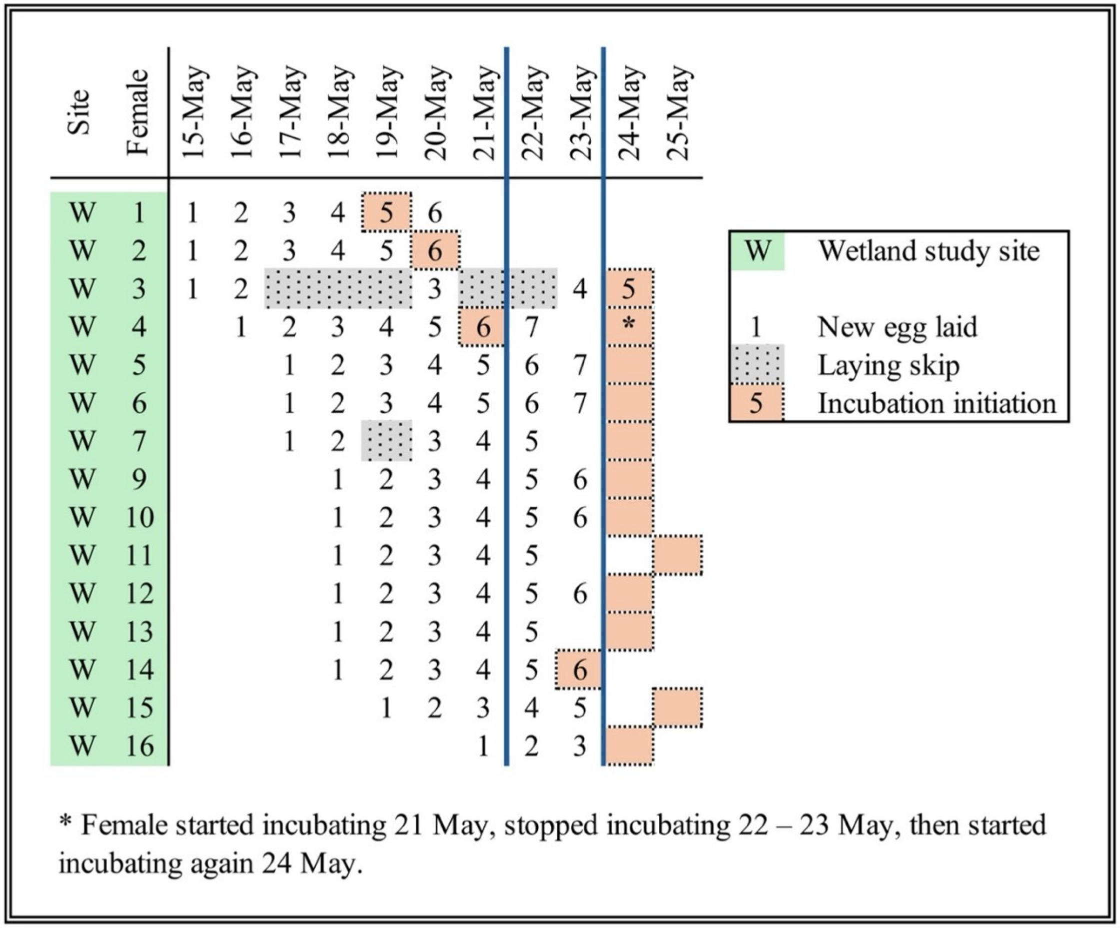
Table 4.2. Results of final generalized linear mixed models testing the effects of female quality, site conditions, and lay-to-set interval (number of days between when an egg is laid and when incubation begins) on the mass and viability of eggs laid by tree swallows breeding near Prince George, British Columbia, Canada, in 2023. Models were run to test the following response variables: A) Mass of eggs laid following a cold snap by females who were undergoing the critical period of rapid yolk development (RYD; two days prior to egg laying) for at least one egg during the cold snap; B) Embryonic viability (hatched or failed to hatch) of all eggs that may have been affected by the cold snap (i.e., were being incubated, awaiting the onset of incubation, or were undergoing RYD during the cold snap); C) Embryonic viability of eggs laid by females that may have responded to the cold snap with a laying snap (i.e., at least one egg in each clutch was undergoing RYD during the cold snap). The z-value is reported for the binomial regressions of embryonic viability, and the t-value is reported for the linear regression of egg mass. Female identity was included as a random effect in all models. Bolded P-values are considered significant at alpha = 0.05.

	β ± SE	t or z-value	P
A) Post-cold snap egg mass (n = 181)			
Laying order	0.08 ± 0.05	1.81	0.07
Max temp T-2[1] (ºC)	0.24 ± 0.06	3.73	**< 0.001**
Laid by female with a laying skip (yes/no)[2]	0.78 ± 0.37	2.11	**0.04**
B) Embryonic viability (n = 192)			
Clutch initiation date	0.77 ± 0.37	2.07	**0.04**
C) Embryonic viability (n = 186)			
Lay-to-set interval (days)	-0.18 ± 0.10	-1.86	0.06

[1] Maximum temperature two days prior to egg laying.

[2] Results are displayed for “yes” in reference to “no”.

4.4.4 Figures

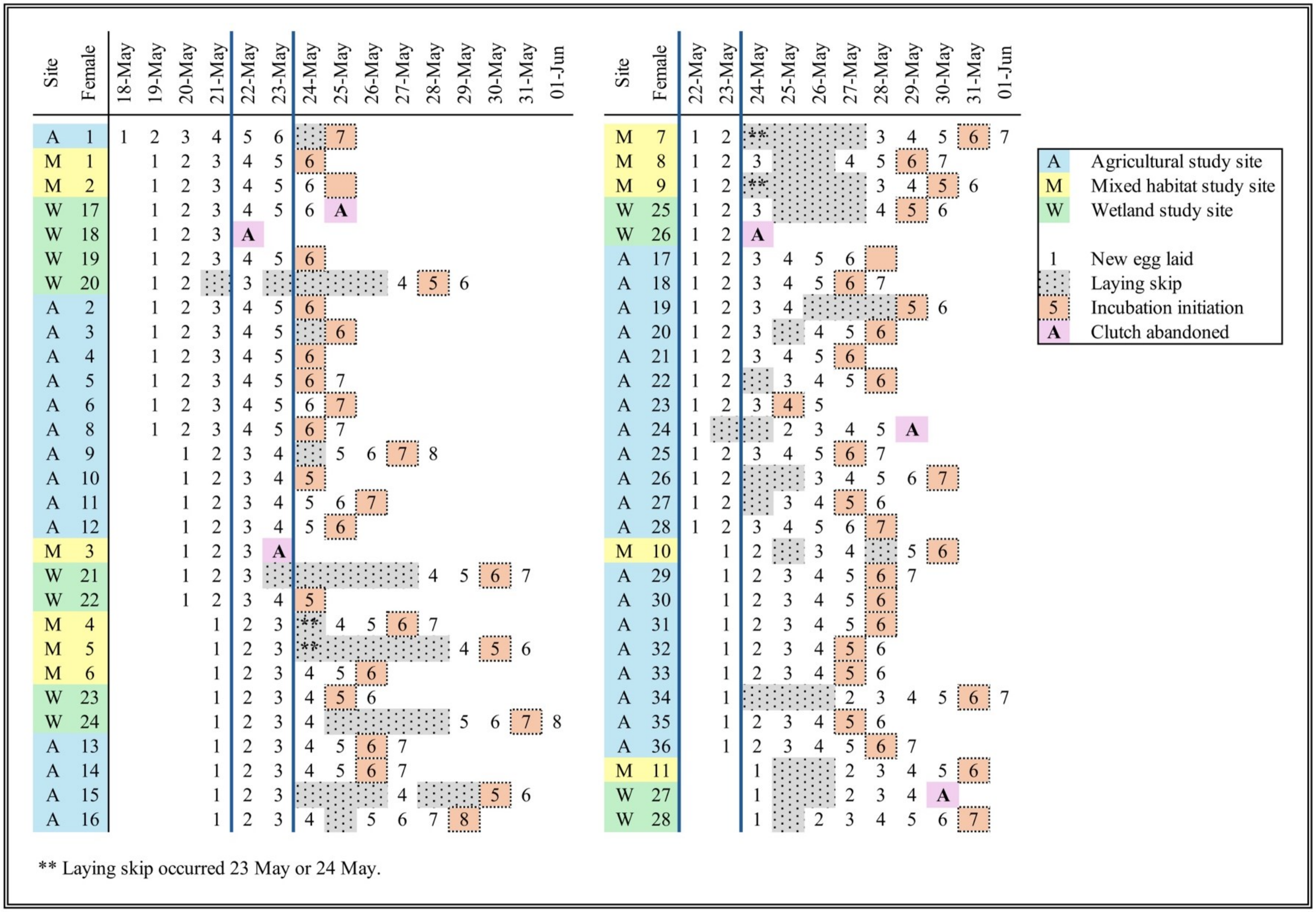


Site	Female	15-May	16-May	17-May	18-May	19-May	20-May	21-May	22-May	23-May	24-May	25-May
W	1	1	2	3	4	🟧 5	6					
W	2	1	2	3	4	5	🟧 6					
W	3	1	2	▨	▨	▨	3	▨	▨	4	🟧 5	
W	4		1	2	3	4	5	🟧 6	7		🟧 *	
W	5			1	2	3	4	5	6	7	🟧	
W	6			1	2	3	4	5	6	7	🟧	
W	7			1	2	▨	3	4	5		🟧	
W	9				1	2	3	4	5	6	🟧	
W	10				1	2	3	4	5	6	🟧	
W	11				1	2	3	4	5			🟧
W	12				1	2	3	4	5	6	🟧	
W	13				1	2	3	4	5		🟧	
W	14				1	2	3	4	5	🟧 6		
W	15					1	2	3	4	5		🟧
W	16							1	2	3	🟧	

W	Wetland study site
1	New egg laid
▨	Laying skip
🟧 5	Incubation initiation

* Female started incubating 21 May, stopped incubating 22 – 23 May, then started incubating again 24 May.

Figure 4.1. Eggs laid by tree swallows who completed their clutches before or during a cold snap (17:00 hr 21 May – 08:00 hr 24 May) that occurred during the egg laying period in 2023 near Prince George, British Columbia, Canada. Three study sites were included in this study (wetland, agricultural, and mixed habitat), but no females in the agricultural or mixed habitat study sites completed their clutches before or during the cold snap. Of the 14 females expected to initiate incubation during the cold snap (W-3 to W-16), 12 (92.86%) delayed incubation until 24 or 25 May.



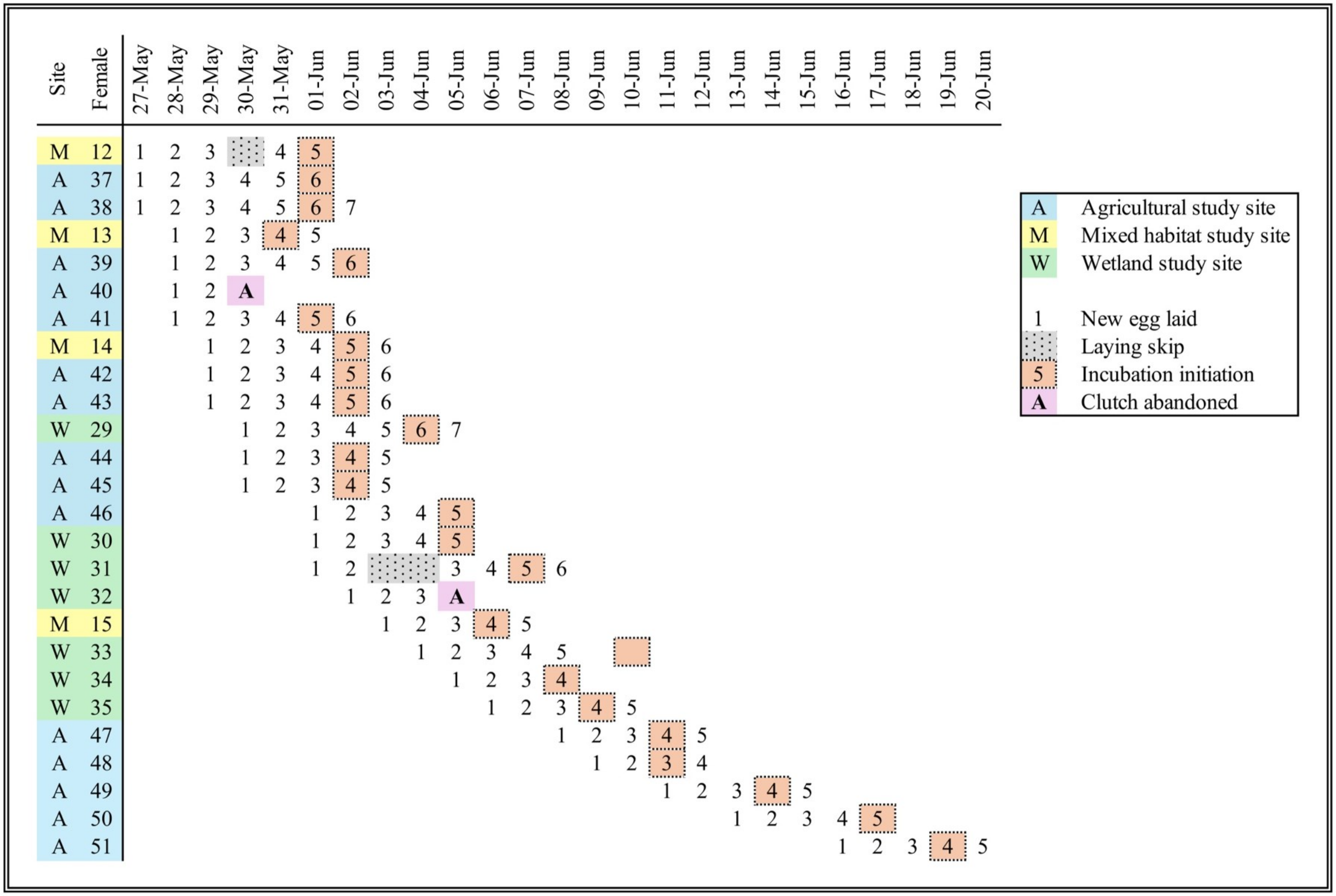
Site	Female	18-May	19-May	20-May	21-May	22-May	23-May	24-May	25-May	26-May	27-May	28-May	29-May	30-May	31-May	01-Jun
A	1	1	2	3	4	5	6	▨	🟧7							
M	1		1	2	3	4	5	🟧6								
M	2		1	2	3	4	5	6	🟧							
W	17		1	2	3	4	5	6	**A**							
W	18		1	2	3	**A**										
W	19		1	2	3	4	5	🟧6								
W	20		1	2	▨	3	▨	▨	▨	▨	4	🟧5	6			
A	2		1	2	3	4	5	🟧6								
A	3		1	2	3	4	5	▨	🟧6							
A	4		1	2	3	4	5	🟧6								
A	5		1	2	3	4	5	🟧6	7							
A	6		1	2	3	4	5	6	🟧7							
A	8		1	2	3	4	5	🟧6	7							
A	9			1	2	3	4	▨	5	6	🟧7	8				
A	10			1	2	3	4	🟧5								
A	11			1	2	3	4	5	6	🟧7						
A	12			1	2	3	4	5	🟧6							
M	3			1	2	3	**A**									
W	21			1	2	3	▨	▨	▨	▨	▨	4	5	🟧6	7	
W	22			1	2	3	4	🟧5								
M	4				1	2	3	▨**	4	5	🟧6	7				
M	5				1	2	3	▨**	▨	▨	▨	▨	4	🟧5	6	
M	6				1	2	3	4	5	🟧6						
W	23				1	2	3	4	🟧5	6						
W	24				1	2	3	4	▨	▨	▨	▨	5	6	🟧7	8
A	13				1	2	3	4	5	🟧6	7					
A	14				1	2	3	4	5	🟧6	7					
A	15				1	2	3	▨	▨	▨	4	▨	▨	🟧5	6	
A	16				1	2	3	4	▨	5	6	7	🟧8			

Site	Female	22-May	23-May	24-May	25-May	26-May	27-May	28-May	29-May	30-May	31-May	01-Jun
M	7	1	2	▨**	▨	▨	▨	3	4	5	🟧6	7
M	8	1	2	3	▨	▨	4	5	🟧6	7		
M	9	1	2	▨**	▨	▨	▨	3	4	🟧5	6	
W	25	1	2	3	▨	▨	▨	4	🟧5	6		
W	26	1	2	**A**								
A	17	1	2	3	4	5	6	🟧				
A	18	1	2	3	4	5	🟧6	7				
A	19	1	2	3	4	▨	▨	▨	🟧5	6		
A	20	1	2	3	▨	4	5	🟧6				
A	21	1	2	3	4	5	🟧6					
A	22	1	2	▨	3	4	5	🟧6				
A	23	1	2	3	🟧4	5						
A	24	1	▨	▨	2	3	4	5	**A**			
A	25	1	2	3	4	5	🟧6	7				
A	26	1	2	▨	▨	3	4	5	6	🟧7		
A	27	1	2	▨	3	4	🟧5	6				
A	28	1	2	3	4	5	6	🟧7				
M	10		1	2	▨	3	4	▨	5	🟧6		
A	29		1	2	3	4	5	🟧6	7			
A	30		1	2	3	4	5	🟧6				
A	31		1	2	3	4	5	🟧6				
A	32		1	2	3	4	🟧5	6				
A	33		1	2	3	4	🟧5	6				
A	34		1	▨	▨	▨	2	3	4	5	🟧6	7
A	35		1	2	3	4	🟧5	6				
A	36		1	2	3	4	5	🟧6	7			
M	11			1	▨	▨	2	3	4	5	🟧6	
W	27			1	▨	▨	2	3	4	**A**		
W	28			1	▨	2	3	4	5	6	🟧7	

A	Agricultural study site
M	Mixed habitat study site
W	Wetland study site
1	New egg laid
▨	Laying skip
🟧5	Incubation initiation
A	Clutch abandoned

** Laying skip occurred 23 May or 24 May.

Figure 4.2. Eggs laid by tree swallows who initiated their clutches before or during, and completed their clutches after, a cold snap (17:00 hr 21 May – 08:00 hr 24 May) that occurred during the egg laying period in 2023 near Prince George, British Columbia, Canada. Six clutches were abandoned before incubation was initiated. Of the 52 clutches that were incubated, 23 (44.23%) were laid by females that had at least one laying skip. No females initiated incubation during the cold snap.



Site	Female	27-May	28-May	29-May	30-May	31-May	01-Jun	02-Jun	03-Jun	04-Jun	05-Jun	06-Jun	07-Jun	08-Jun	09-Jun	10-Jun	11-Jun	12-Jun	13-Jun	14-Jun	15-Jun	16-Jun	17-Jun	18-Jun	19-Jun	20-Jun
M	12	1	2	3	▨	4	🟧 5																			
A	37	1	2	3	4	5	🟧 6																			
A	38	1	2	3	4	5	🟧 6	7																		
M	13		1	2	3	🟧 4	5																			
A	39		1	2	3	4	5	🟧 6																		
A	40		1	2	**A**																					
A	41		1	2	3	4	🟧 5	6																		
M	14			1	2	3	4	🟧 5	6																	
A	42			1	2	3	4	🟧 5	6																	
A	43			1	2	3	4	🟧 5	6																	
W	29				1	2	3	4	5	🟧 6	7															
A	44				1	2	3	🟧 4	5																	
A	45				1	2	3	🟧 4	5																	
A	46						1	2	3	4	🟧 5															
W	30						1	2	3	4	🟧 5															
W	31						1	2	▨	▨	3	4	🟧 5	6												
W	32							1	2	3	**A**															
M	15								1	2	3	🟧 4	5													
W	33									1	2	3	4	5		🟧										
W	34										1	2	3	🟧 4												
W	35											1	2	3	🟧 4	5										
A	47													1	2	3	🟧 4	5								
A	48														1	2	🟧 3	4								
A	49																1	2	3	🟧 4	5					
A	50																		1	2	3	4	🟧 5			
A	51																					1	2	3	🟧 4	5

A	Agricultural study site
M	Mixed habitat study site
W	Wetland study site
1	New egg laid
▨	Laying skip
🟧 5	Incubation initiation
A	Clutch abandoned

Figure 4.3. Eggs laid by tree swallows who initiated clutches following a cold snap (17:00 hr 21 May – 08:00 hr 24 May) in 2023 near Prince George, British Columbia, Canada. No clutches were initiated 25 – 26 May. Two clutches were abandoned before they were completed. Of the 23 remaining clutches, 2 (8.70%) were laid by females who had laying skips, and 1 (4.35%) was laid by a female who delayed incubation. All other 20 (86.96%) clutches were laid by females who did not have laying skips and who initiated incubation after laying their penultimate or final eggs.

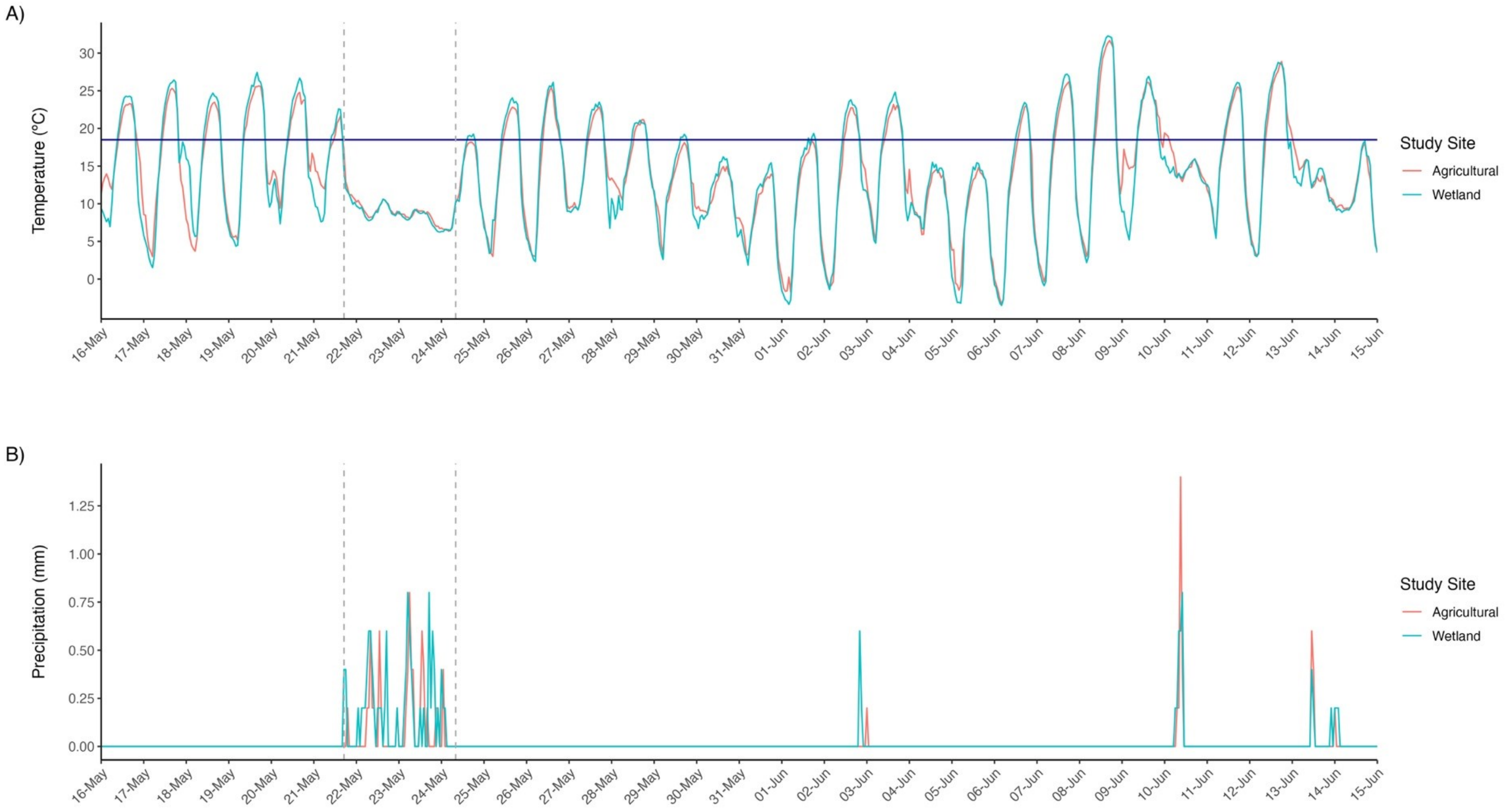


Figure 4.4. Mean hourly temperature (ºC; A) and precipitation (mm; B) during the 2023 breeding season at two study sites near Prince George, British Columbia, Canada. Grey dashed lines indicate the start (17:00 hr, 21 May) and end (08:00 hr, 24 May) of a cold snap that occurred during the egg laying period of tree swallows breeding at the study sites. The solid line on the y-axis of temperature represents a critical daily maximum temperature (18.5 ºC) for insect flight, below which tree swallows are known to experience juvenile mortality during the nestling period (Winkler et al., 2013).

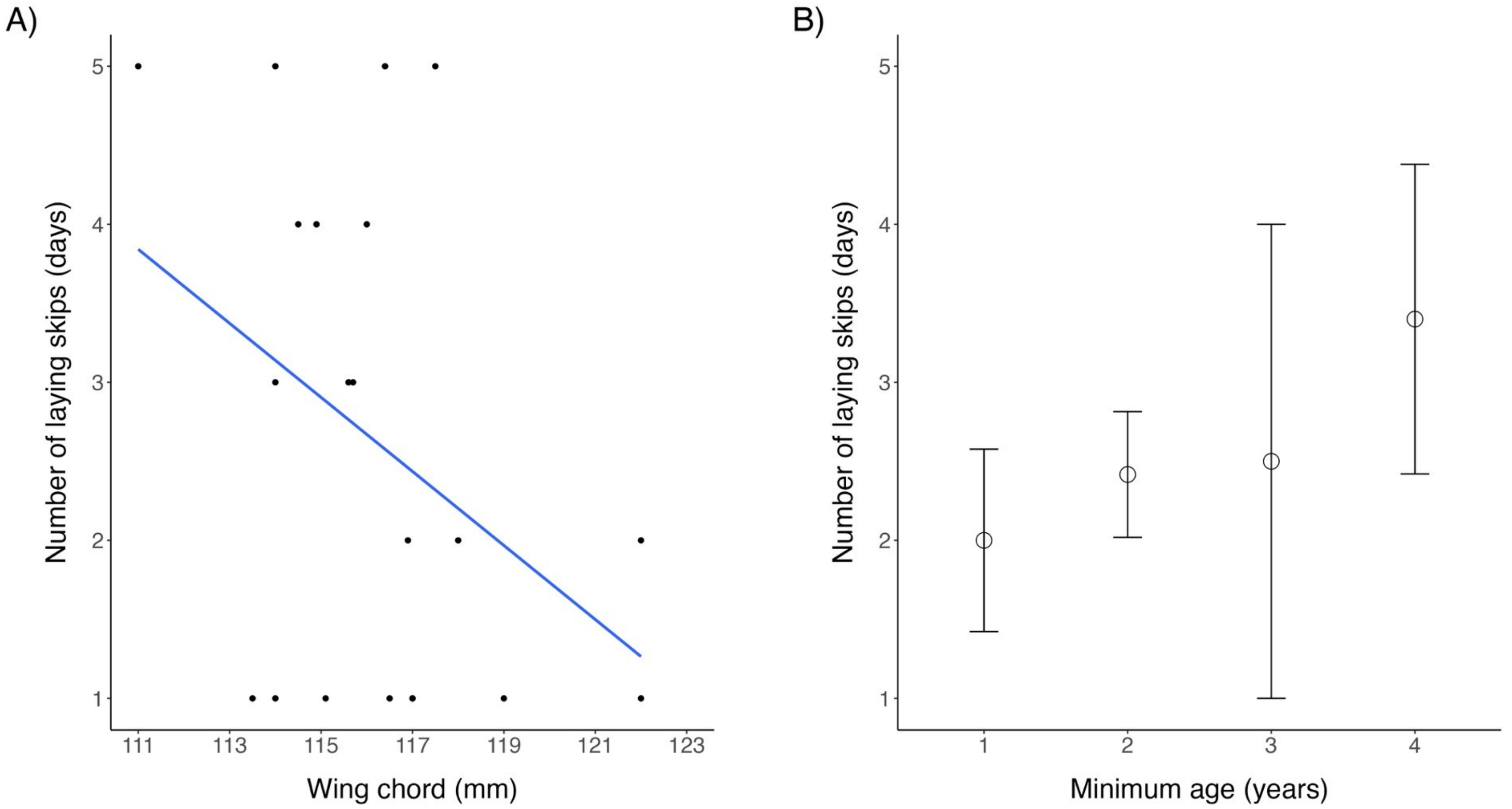


Figure 4.5. The number of laying skips of female tree swallows who had at least one laying skip and initiated their clutches before or during, and completed their clutches after, a cold snap (17:00 hr 21 May – 08:00 hr 24 May) that occurred during the egg laying period in 2023 near Prince George, British Columbia, Canada. The number of laying skips decreased as wing chord length increased and increased as minimum age increased, although the latter trend was not significant (see Table 4.1).

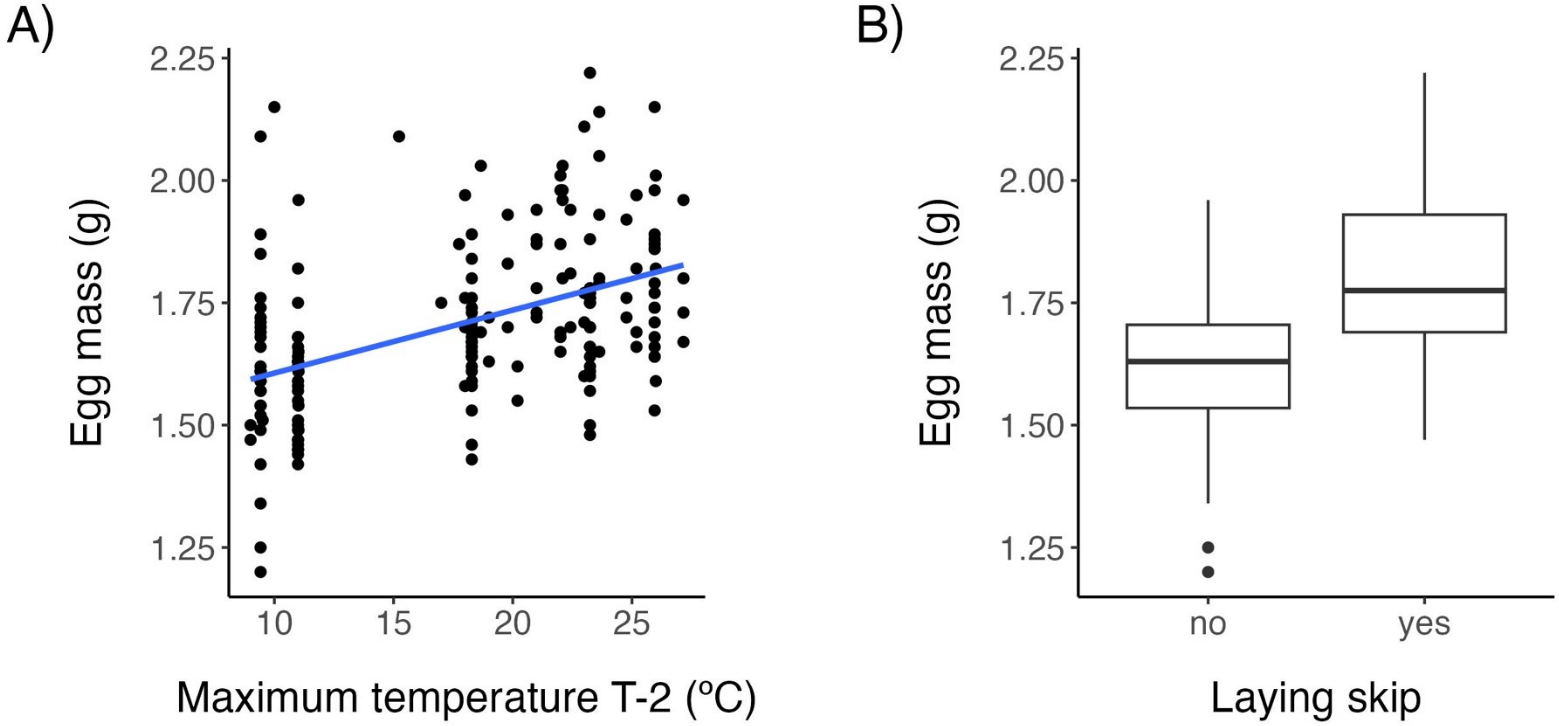


Figure 4.6. The mass of eggs laid after a cold snap (17:00 hr 21 May – 08:00 hr 24 May) by tree swallows breeding near Prince George, British Columbia, Canada, in 2023 who had at least one laying skip and initiated their clutches before or during, and completed their clutches after, the cold snap. Egg mass increased as maximum temperature two days prior to egg laying increased (A; $\beta = 0.24 \pm 0.06$, $t = 3.73$, $P < 0.001$), and eggs laid by females with at least one laying skip were heavier than eggs laid by females without laying skips (B; $\beta = 0.78 \pm 0.37$, $t = 2.11$, $P = 0.04$).

4.5 DISCUSSION

The cold snap that occurred in and around the Prince George region on 22 – 23 May in 2023 was characterized by a precipitous decrease in ambient maximum temperature and occurrence of rainfall following days of warm and dry conditions. Because the abundance of flying insects commonly consumed by tree swallows is higher at moderate temperatures (Garrett et al., 2022) and drops abruptly when ambient temperature declines (Shipley et al., 2020), prey availability was probably low during a critical period in the breeding season when females were forming and laying eggs. These conditions provided a unique opportunity to assess the effects of a cold snap on prenatal reproductive investment by female tree swallows within both their physiological (i.e., laying skips and egg quality) and their behavioural (i.e., the onset of incubation) responses to the cold snap.

4.5.1 Laying skips

Laying skips have been associated with cold weather, spring storms, and low food availability in various wild avian species and study systems (Eeva & Lehikoinen, 2009; Lessells et al., 2022; Monrós et al., 1998; Nilsson & Svensson, 1993; Wiebe & Martin, 1995). Nearly half of females who were actively laying during the cold snap appeared to respond to the cold snap with at least one laying skip. Despite extending lay-to-set intervals of earlier-laid eggs, laying skips were not directly detrimental to reproductive success; eggs laid by females with laying skips had the same odds of containing a viable embryo as eggs laid by females without laying skips, and females who had laying skips laid heavier eggs after the cold snap. No common measures of female quality in tree swallows, such as minimum age, body condition, feather reflectance, or clutch initiation date, were related to the occurrence of laying skips. This finding is consistent with research conducted on other wild species where laying skips were not associated with maternal body condition, corticosterone levels during the egg laying period, age, or laying date (Henderson et al., 2014; Nilsson & Svensson, 1993; Wiebe & Martin, 1995). Laying skips may therefore provide some females an opportunity to replenish resources required for egg laying without compromising the hatching success of their embryos, but the females that require at least one skipped day in the laying cycle are apparently of no lesser quality than females who continue laying following poor environmental conditions.

Females with laying skips laid larger clutches, which may indicate that laying skips provide an opportunity for females to lay more eggs. Alternatively, this trend could occur if females pre-destined to lay larger clutches are more likely to have laying skips in response to cold snaps. Smaller clutches within tree swallow populations have been linked to lower food abundance (Hussell & Quinney, 2008), lighter maternal body mass (Pellerin et al., 2015), site contamination (Dods et al., 2005), and agricultural intensity (Ghilain & Bélisle, 2008). Additionally, females with experimentally clipped feathers, and therefore assumed lower foraging efficacy, laid smaller clutches than females in control groups in two separate studies (Nooker et al., 2005; Winkler & Allen, 1995). Clutch size could therefore be viewed as an indicator of female quality and reproductive success, and laying skips may allow females to replenish the resources they require to lay larger clutches.

Because laying skips occurred more frequently during the earlier part of the laying sequence of a clutch and females skipped up to five days, laying skips in this population are more likely to be caused by follicular atresia, rather than misalignments with a female's open period, based on the current understanding of these mechanisms (reviewed in Williams, 2012). It is possible, however, that some females may have experienced delayed maturation of their F1 follicle during the cold snap due to decreased prey abundance, and the follicle could not be ovulated until the following day. As egg mass is closely associated with maximum temperature two days before laying and the follicle is ovulated one day before laying, a laying skip that resulted from delayed maturation of the F1 follicle during the cold snap would most likely have occurred on 23, 24, or 25 May. Ten of the 23 females with laying skips seemingly associated with the cold snap had a single laying skip within the 23 – 25 May time period. Additionally, follicular atresia is not known to occur in the F1 or F2 follicles of passerines, although very little research has been done on this topic (Challenger et al., 2001). The proximate cause of laying skips in this population could therefore be a combination of delayed maturation of the F1 or F2 follicles in some females and follicular atresia in others. The factors that predict incidence of laying skips may not be detected if both causes occurred in this population and are linked to different variables. It was not possible, however, to assess the follicular structure of females within the scope of this study, so all females with laying skips following the cold snap remained pooled together for analyses.

Females with shorter wings had more laying skips, and there was a trend for older females to have more laying skips following the cold snap. While skipped days were usually sequential, a few females (e.g., A-15 and M-10; Figure 4.3) had multiple laying skips interspersed with egg laying. Females with shorter wings may be limited in foraging ability, and females with experimentally shortened wing feathers delay initiating their clutches until later in the year (Winkler & Allen, 1996). Past research on the quality of adult tree swallows indicates older individuals have longer wings (Randall & Dawson, unpublished data), but age and wing chord length were not related in this study. This could be due to the smaller sample size of this study compared to longer-term datasets. The delayed plumage maturation of SY female tree swallows presents an opportunity to accurately age females in their first breeding season, even when individuals are recruited from other unbanded populations, and researchers often categorize females as SY or ASY to investigate the differences between them. Typically, ASY females are superior to their SY counterparts, as they have a higher body mass, initiate breeding earlier, lay larger eggs and clutch sizes, raise larger nestlings, and fledge more young (Ardia et al., 2006c; Beck et al., 2015; Bentz & Siefferman, 2013; Bitton & Dawson, 2017; Winker & Allen, 1996). Notably, rather than group females into SY and ASY categories, I assigned a minimum age to each female based on a combination of plumage colour and banding information, and used age as a continuous variable in my models. Tree swallows only live 2 – 3 years on average (Winkler et al., 2020a), so the older females in this population, aged up to at least six years old, may be unable to undergo RYD as rapidly as younger females due to natural senescence. This aging effect has been observed in domestic poultry (Joyner et al., 1987), but little is known about the relationship between senescence and egg production in passerines.

The ornamentation of female plumage did not predict the occurrence or number of laying skips in this study. The relationship between plumage characteristics and adult quality in tree swallows is complex. Brighter and bluer feathers have been shown as important for various aspects of breeding, such as conspecific competition between females (Beck & Hopkins, 2019; Berzins & Dawson, 2016; Coady & Dawson, 2013), assortative mating (Bitton et al., 2008), and extrapair fertilization success by males (Bitton et al., 2007; Whittingham & Dunn, 2016). Additionally, brighter females have been associated with clutches with higher mean egg masses and greater fledging success, and bluer females also fledge more young (Berzins & Dawson, 2016; Bitton et al., 2008). Griebel & Dawson (2019), however, found that nestlings were less likely to survive a cold snap when they were cared for by females with brighter and more UV-reflective plumage. Bentz and Siefferman (2013) found that bluer and brighter females laid larger eggs but had lower immune responses and raised offspring in lower body condition, but these results are not directly comparable to other tree swallow research as Bentz and Siefferman (2013) did not include UV chroma in the PCA of feather colour traits and therefore only tested one principal component combining brightness, blue chroma, and hue. Differences in results between these studies and mine could be due to statistical design, geographic area, or other co-varying factors of feather ornamentation as a signal of reproductive success that have yet to be explored. Additionally, while plumage characteristics may be important for other aspects of breeding, these traits may not be important signals related to the maintenance of RYD following adverse environmental conditions.

Females breeding at the agricultural study site were less likely to experience laying skips after the cold snap, and those that did have laying skips experienced skips for fewer days in comparison to females breeding at the mixed habitat and wetland study sites. Peak insect activity at the agricultural site occurred 17 – 20 May in 2023 and was associated with a high abundance of March flies (*Bibionidae*; order Diptera, suborder Nematocera; Appendix A). This particular mass emergence of flies occurred immediately preceding the cold snap and was not observed at either of the other two study sites (pers. obs.). Females at the agricultural study site may have therefore been more likely to have greater foraging success and receive sufficient resources to maintain RYD and associated egg production through the cold snap. These results emphasize the importance of studying the timing of breeding insectivorous birds with diet availability at a site-specific level, as localized populations of insects differ in community composition and their response to environmental conditions (Dunn et al., 2023).

4.5.2 Egg quality

Following the cold snap, eggs laid by females with at least one laying skip were heavier than eggs laid by females who did not skip laying, and this relationship is consistent with findings from other wild avian populations (Cucco et al., 2017; Kennamer et al., 1997; Nilsson & Svensson, 1993). The maximum daily temperature two days prior to laying date was a significant predictor of egg mass, both in the final model and in the preliminary analysis of climate timing windows. This finding is consistent with Ardia et al. (2006c) who found that insect availability two to three days prior to egg laying predicted whole mass of 585 eggs, as maximum daily temperature is strongly correlated with the abundance of insect prey of aerial insectivores (Garrett et al., 2022). Delaying egg laying by skipping days after the cold snap provided females with an opportunity to take advantage of warmer days during the critical RYD period, as well as greater absolute time to synthesize the proteins, hormones, and nutrients required for egg production.

Although eggs laid by females with laying skips were heavier, neither egg mass nor the laying skip status of a female (i.e., whether or not they had at least one laying skip) predicted embryonic viability of eggs laid after the cold snap. While a few studies of wild birds have detected a positive relationship between egg mass and hatching success (e.g., Perrins, 1996; Sanchez-Lafuente, 2004), various others have found no effect (e.g., Beissinger et al., 2005; Magrath, 1992; Viñuela, 1997), including a recent study of tree swallows and 13 other passerine species in Illinois (Di Giovanni et al., 2022). Lombardo et al. (2021) detected a positive trend between hatching success and egg mass in tree swallows, but only in eggs laid by SY females, and the effect size was small. A complicating factor to consider when evaluating egg mass is that maternal investment is allocated differentially among the yolk, albumen, and shell components, and while the majority of nutrients and hormones essential for embryos are located in the yolk, albumen mass is closely associated with embryonic size and growth (Deeming, 2002). The assumption that larger egg mass is advantageous may fail to account for the composition and concentrations of key resources for embryos in yolks; therefore, a cautious approach should be used when considering the effects of whole egg size as a measure of reproductive success.

When all eggs that may have been affected by the cold snap were evaluated, embryonic viability increased as clutches were initiated later in the breeding season. In this timeframe, the latest clutches were initiated during the cold snap, so it is possible that eggs that were laid before the cold snap and were either being incubated or waiting for incubation through the colder temperatures were less likely to hatch than eggs that were formed during the cold snap. To investigate this possibility, I assigned eggs in this dataset a categorical variable indicating if they were laid before or during the cold snap, or if they were formed during the cold snap and laid immediately after the cold snap. I tested if this variable predicted embryonic viability using a binomial regression with female identity as a random effect and found the relationship was not significant ($\beta = -0.81 \pm 0.65$, $z = -1.25$, $P = 0.21$). Furthermore, the laying date of individual eggs was not a significant factor in the initial model set, and the positive relationship between clutch initiation date and embryonic viability remained significant when eggs laid at the agricultural and wetland sites in both 2022 and 2023 were assessed together (Chapter 3). This effect may therefore have persisted in spite of, and be unrelated to, the cold snap during the egg laying period of 2023.

In tree swallows, the relative date a female lays her first egg is indicative of many measures of individual quality and reproductive success, including eggs with larger yolks, larger clutch sizes, higher nestling survival, and higher recruitment (Ardia et al., 2006c; Berzins et al., 2020; Dunn et al., 2011; Shutler et al., 2006; Stutchbury & Robertson, 1988). Additionally, pairs with more combined breeding experience initiate egg laying earlier (Lombardo & Thorpe, 2010), which may be because they have site fidelity and are able to acquire nesting sites with more desirable microclimates (Ardia et al., 2006b). Accordingly, embryos of higher quality females may be expected to hatch more often, especially given that production of unhatched eggs represent a loss of maternal investment (Williams, 2005). Although female tree swallows who lay eggs earlier in the breeding season are qualitatively superior, embryonic development may be deleteriously affected by factors during this period, such as the nutritional content of insect prey (Twining et al., 2018), that I did not measure in this study. Because earlier-breeding adults fledge more young (Dunn et al., 2011), there may be a trade-off between hatching success and juvenile survival, although Nooker et al. (2005) found that the number of eggs that hatched in a clutch increased fledging success.

4.5.3 Delayed incubation and extended lay-to-set intervals

Most females that were expected to begin incubating during the cold snap (22 – 23 May) delayed the onset of incubation until 24 – 25 May. Tree swallows usually initiate incubation immediately after laying their penultimate or final eggs, and delayed incubation is not normally observed in this species when environmental conditions are within expected ranges (Lombardo, 1995; Zach, 1982). Female tree swallows are more likely to begin incubating earlier (i.e., one day prior to clutch completion) when temperatures during laying are warmer (Ardia et al., 2006a), presumably to maintain egg viability, but delay incubation during periods of increased precipitation (Wang & Beissinger, 2009). Incubation is energetically costly (reviewed in Nord & Williams, 2015), so delaying incubation during inclement weather may be an indication that females are energetically constrained at these times.

Laying skips, delayed incubation, and larger clutch sizes are all factors that can extend lay-to-set intervals, particularly of earlier-laid eggs. Eggs laid by females in Group 2 with longer lay-to-set intervals had lower viability, although the relationship only approached significance. The number of inviable eggs included in this analysis was relatively low and the distribution of lay-to-set intervals was naturally skewed toward lower intervals, so it is difficult to determine if the trend is biologically relevant. As tree swallows rarely lay more than seven eggs per clutch, the first-laid egg in a large clutch usually will not remain unincubated for more than six days if the female waits until laying her final egg to begin incubation. Lay-to-set interval may not be important, therefore, if females do not lay exceptionally large clutches and begin incubating as expected. Viability may only begin to decline once lay-to-set intervals exceed a higher number of days than detected in this study, which is consistent with research on other species. In domestic chickens, the viability of eggs stored for 7 or fewer days did not differ from eggs incubated immediately, but significantly declined after storage of 8 or more days (reviewed in Fasenko, 2007), and Bollinger et al. (2018) found that the viability of eastern bluebird (*Sialis sialis*) eggs stored indoors began to decrease substantially after 4 days.

A complicating factor in evaluating lay-to-set intervals is that females may engage in partial incubation, in which eggs laid earlier in the clutch are temporarily warmed to incubation temperatures before the clutch is completed and full incubation begins. Partial incubation is common in many species, including tree swallows, and often begins shortly after the first or second egg is laid (Johnson et al., 2013; Wang & Beissinger, 2009; Wang & Beissinger, 2011). Partial incubation of just a few hours is known to increase the viability of eggs stored for longer periods of time (Fasenko, 2007), so females in my study may have been able to preserve the viability of their eggs through the cold snap by briefly incubating them until their clutches were complete. Notably, the two eggs with the longest lay-to-set interval of 10 days both hatched successfully.

4.5.4 Plasticity of tree swallows in response to climatic variability

Clutch initiation dates of tree swallows and many other avian species have advanced significantly in the past 50 years, and this phenomenon has been linked to climate warming (Dunn, 2019). Warming temperatures can also advance hatching dates, as not only is laying initiated earlier, but warmer temperatures during egg laying shorten incubation periods (e.g., Ardia et al., 2006a; Vedder, 2012). Species with advancing lay dates are at risk of experiencing a phenological mismatch in which young hatch earlier than peak foraging periods, known as the "temporal mismatch hypothesis" (reviewed in Durant et al., 2007). A phenological mismatch between hatching dates and insect prey abundance has not yet been observed in tree swallows (Dunn et al., 2011); however, flying insects respond differently to changes in temperature across North America (Dunn et al., 2023), so an evaluation of the temporal mismatch hypothesis at a site-specific level may be required to detect a mismatch between local tree swallow and insect prey populations. Furthermore, the incidence of harsh weather events during chick rearing has increased, leading to sudden declines in flying insects on cold days and associated mass chick mortalities (Shipley et al., 2020).

Conversely, the prevalence of laying skips and delayed incubation in response to colder temperatures can result in hatching delays. Hatching delays increase the total time adults spend caring for their young in the nest, either as embryos or nestlings, which may increase predation risk. If breeding adults are able to avoid depredation however, hatching delays may be a beneficial behavioural adjustment to variability in environmental conditions. Hatching delays do not decrease overall fitness (Monrós et al., 1998) and may ultimately result in hatching dates that are better timed with food availability and ideal temperatures for nestling survival (Tomás, 2015). Instances of variable weather continued to occur throughout the nestling period at my study sites in 2023, and a cold snap from 30 – 31 May, as well as depredation events at the agricultural and wetland sites, resulted in large incidences of chick mortality and I was unable to assess fledging success in relation to the cold snap during the egg laying period.

The detrimental effects of cold snaps on tree swallow nestlings have been well documented (e.g., Cox et al., 2020; Garrett et al., 2022; Griebel & Dawson, 2019). Winkler et al. (2013) determined there were two critical temperature thresholds for insect flight activity at their study sites in Ithaca, New York, at 15.5 ºC and 18.5 ºC, and further defined cold snaps in the nestling period as consecutive days in which the maximum temperature did not exceed 18.5 ºC. This critical threshold has been used to identify cold snaps in subsequent research on tree swallows and has been directly related to nestling mortality (Shipley et al., 2020). The threshold for which ambient temperature begins to affect egg laying, and the extent to which egg laying can be affected, has yet to be determined. The maximum temperature during the 30 – 31 May cold snap was 17.8 ºC, and while chick mortality during and immediately following this period was evident (pers. obs.), females that were laying eggs at this time did not delay incubation and only one female had at least one laying skip following the cold snap. The energy required for RYD and incubation is costly, particularly for tree swallows as the males do not incubate or forage for females, but females may be able to acquire sufficient resources needed for self-maintenance, egg formation, and provisioning of their embryos at lower temperatures than when they are providing parental care for nestlings. It is therefore possible that the critical threshold that may be used to identify a cold snap during the egg laying period is closer to 15.5 ºC than the 18.5 ºC threshold for nestlings. Further work is required to determine the environmental conditions at which females begin to delay incubation and experience laying skips, and the thresholds at which embryonic viability will begin to decline.

As climate change continues to increase the occurrence of harsh weather events, longer-term research on the effects of climate variability on tree swallows and other migratory passerines will be important in determining how species dependent on cyclic temperature patterns can adapt into the future. Case studies such as this one are naturally limited in sample size and scope; however, evaluating the effects of a single cold snap provides an opportunity to observe how a local population responds to a specific weather event. This study provides insight into how birds dependent on short-term availability of resources, such as aerial insectivores, respond to sudden fluctuations in environmental conditions during the prenatal investment period.

Chapter 5: General discussion

Eggs are the first form of maternal investment birds allocate to their young, as the internal contents provide the resources necessary for the growth and hatching of embryos (Carey, 1996). The formation, laying, and incubation of eggs represents a large prenatal investment by female birds, particularly in species in which males do not contribute to incubation or foraging for the female while she is laying or incubating. An unhatched egg therefore represents a large energetic loss to the female. Although up to 17% of avian eggs on average do not hatch, relatively little is known about the causes of hatching failure (reviews by Koenig, 1982, and Marshall et al., 2023). To fully assess hatching failure, however, it is important to understand if hatching failure is due to infertility or embryonic mortality. Microscopic methods to differentiate between early embryonic mortality and infertility have been underused by researchers, resulting in an overestimation of infertility, or an inability to infer reasons for prenatal mortality in avian populations (Hemmings & Evans, 2020).

The objective of my study was to understand the causes of hatching failure in tree swallows, a species which has been widely studied across North America, yet little is known about fertility rates or causes of hatching failure. I also aimed to explore the extrinsic (e.g., environmental conditions) and intrinsic (e.g., female quality) factors related to prenatal reproductive success in tree swallows. I achieved these objectives by 1) quantifying fertility and the developmental stages of embryonic mortality within the population, and 2) assessing factors influencing prenatal reproductive success in tree swallows. Additionally, I opportunistically explored the effects of a cold weather event during the egg laying period of a single breeding season on maternal plasticity of incubation behaviour, egg laying, and embryonic viability.

Through microscopic fertility testing, I determined that no eggs laid in my study were conclusively unfertilised, resulting in a minimum fertility rate of 98.3% and a maximum fertility rate of 100% (Chapter 2). This study is the first to my knowledge to quantify fertility and developmental stages of embryonic mortality in tree swallows and demonstrates that hatching failure in this population is therefore predominantly due to embryonic mortality, not infertility. Importantly, I demonstrated that eggs frozen for long periods of time, and in sub-optimal conditions (i.e., experiencing a freeze-thaw-freeze event) could successfully undergo microscopic fertility testing. This finding provides an opportunity for research and conservation groups with long-term monitoring programs to investigate the fertility of their respective species if 1) programs have been keeping unhatched eggs in frozen storage, or 2) programs do not currently have the resources to test for fertility immediately and wish to freeze unhatched eggs for future testing. Because only the germinal disc is required for a conclusive test, fertility testing can be paired with other laboratory tests that do not require the entire egg. Furthermore, I found that preparing multiple slides of disintegrated pieces of perivitelline layer for microscopy usually resulted in finding disintegrated portions of germinal disc as well. Thousands of embryonic cells are present in a fertile egg at oviposition (Watt et al., 1993) and many can be successfully detected using a fluorescent stain even if the embryo died early in incubation and the egg was subsequently incubated for more than two weeks, as was the case with some of the eggs tested in this study.

Most embryonic mortality in this study occurred during early incubation (Chapter 2). Early embryonic mortality is common across many avian species (Assersohn et al., 2021; Christensen 2001; Hemmings & Evans, 2020), and high incidences of mortality during early incubation highlight the usefulness of microscopic fertility testing to confidently differentiate between infertility and early embryonic mortality. In this study, 8.13% (86/1058) of eggs may have otherwise been assumed to be infertile if additional fertility testing was not employed. Unexpectedly, incidences of middle and late incubation mortality were similar, which is not consistent with previous observations in other species that indicate mortality associated with late incubation and hatching are generally higher than middle incubation mortality (Assersohn et al., 2021; Romanoff, 1972). While many factors have been linked to the early-stage and late-stage embryonic mortality periods, little is known about the potential causes of middle-incubation mortality and why death during this period of incubation is usually relatively uncommon. (Assersohn et al., 2021; Christensen, 2001). Larger datasets on embryonic mortality are required to establish if the patterns seen in this study are consistent with embryonic mortality of tree swallows across North America.

The maximum daily temperature two days prior to laying date was a significant predictor of egg mass (Chapter 3), indicating insect availability in this time period is likely important for rapid yolk deposition as maximum daily temperature is strongly associated with the insect prey of aerial insectivores (Garrett et al., 2022). Despite overall warmer temperatures in 2023, egg mass was lower when compared to 2022 (Chapter 3), which may be an effect of the cold snap during the egg laying period of 2023. Females who responded to the cold snap in 2023 with at least one laying skip laid heavier eggs than females without laying skips (Chapter 4), but embryonic viability was not related to egg mass in either year (Chapters 3 & 4). Whereas many avian studies have shown positive relationships between heavier eggs and various measures of the growth and survival of young (reviewed by Krist, 2011), evidence of these effects is lacking in tree swallows (Bitton et al., 2006; De Steven, 1978; Griebel & Dawson, 2016; Taff et al., 2022; Wardrop & Ydenberg, 2003; Whittingham et al., 2007).

Furthermore, whereas a few studies of wild birds have detected a positive relationship between egg mass and hatching success (e.g., Perrins, 1996; Sanchez-Lafuente, 2004), various others have found no effect (e.g., Beissinger et al., 2005; Magrath, 1992; Vinuela, 1997), including a recent study of tree swallows and 13 other passerine species in Illinois (Di Giovanni et al., 2022). The assumption that larger and heavier eggs are superior may fail to account for the composition and concentrations of key resources for embryos in yolks; therefore, a cautious approach should be used when considering the effects of whole egg size as a measure of prenatal investment.

Contrary to expectations, embryonic viability increased as clutches were laid later in the breeding season, both in 2022 and 2023 combined (Chapter 3) and in a subset of eggs that were laid before or during the cold snap of 2023 (Chapter 4). Although female tree swallows who lay eggs earlier in the breeding season are qualitatively superior (Ardia et al., 2006c; Dunn et al., 2011; Stutchbury & Robertson, 1988), embryonic development may be deleteriously affected by colder temperatures during this time period, either as a consequence of decreased egg content quality from a lower abundance of insect prey, or inferior incubation conditions. Clutch initiation dates of tree swallows have advanced significantly over the past half-century in response to climate warming (Shipley et al., 2020), so future research with longer-term datasets should aim to assess whether embryonic viability is also related to *absolute* initiation date in addition to *relative* initiation date as I have found in this study.

As climates continue to change at an accelerated rate and weather variability increases (Maibach et al., 2014), sudden harsh weather events during the egg laying period, such as the event that occurred in 2023, may become more common. Nearly half of females responded to this event with at least one laying skip, and females with laying skips were more likely to lay larger clutches (Chapter 4). Additionally, most females expected to begin incubation during the cold snap delayed the onset of incubation until one or two days after the cold snap (Chapter 4). Laying skips, larger clutch sizes, and delayed incubation are all mechanisms that extend lay-to-set intervals, particularly of first-laid eggs. The effect of longer lay-to-set intervals on embryonic viability in this study was inconclusive (Chapter 4), and it is possible that a specific threshold exists after which viability sharply declines, as has been observed in other avian species (Bollinger et al., 2018; Fasenko, 2007). The tree swallows in my study population seemingly had the plasticity necessary to respond to a relatively short cold snap during the egg laying period, but more research is required to understand the limitations to these responses in instances of more extreme weather events.

In addition to egg mass and the occurrence of laying skips, age, feather colour and brightness, study site type (i.e., agricultural, wetland, mixed habitat), and the maximum temperature two days prior to egg lay were all unrelated to embryonic viability (Chapters 3 & 4). There are other factors known to constrain embryonic viability in birds that were not be tested in this study, including genetic and developmental abnormalities (Forstmeier & Ellegren, 2010; Shook et al., 1971), low sperm abundance (Hemmings & Birkhead, 2015), and incubation temperature (Webb, 1987). Because female tree swallows are solo intermittent incubators, the frequency and duration of incubation off-bouts may likewise affect the ability of embryos to hatch. Importantly, embryos at different stages of development are susceptible to different factors; therefore, a dataset containing a larger number of eggs with embryos that died in middle and late stages is required to fully evaluate the effects of environmental conditions and maternal investment during the incubation period.

As many avian species continue to decline globally (Lees et al., 2022), it is becoming increasingly important to understand the drivers of mortality in each life stage. To date, few avian studies have quantified fertility or prenatal mortality rates, likely in part due to the difficulty of distinguishing between infertility and early embryonic mortality. Although some species, such as tree swallows, may not be declining due to problems with infertility, it is vital to collect baseline data so changes in fertility rates can be detected in the future. Recent advancements in avian fertility testing, including my finding that fluorescence microscopy is an appropriate testing tool for eggs kept in long-term frozen storage, provide a relatively simple method to determining fertility and should be considered by researchers and conservationists seeking to better understand the reproductive biology of their respective study species. Future studies in tree swallows and other birds should therefore aim to incorporate prenatal mortality into demographic data to ensure adequate representation of this age class in determining lifetime reproductive success and other similar demographic metrics. Additionally, the primary drivers of embryonic mortality should be assessed to further our understanding of the susceptibility of embryos to changes in climatic and other conditions. Knowledge of the fertility rates and drivers for embryonic mortality in populations of any declining or endangered avian species can subsequently be used to inform conservation management strategies, such as the use of artificial incubation and strategic decisions regarding mate-pairing and wild releases in captive breeding programs (e.g., Bussolini et al., 2023; Digby et al., 2023).

References

Aitken, K. E. H., & Martin, K. (2008). Resource selection plasticity and community responses to experimental reduction of a critical resource. *Ecology*, *89*(4), 971–980. https://doi.org/10.1890/07-0711.1

Aldredge, R. A. (2017). Incubation onset maintains survival of most embryos and growth and survival of late-hatched young. *Animal Behaviour*, *134*, 35–43. https://doi.org/10.1016/j.anbehav.2017.09.022

Alonso-Alvarez, C. (2006). Manipulation of primary sex-ratio: An updated review. *Avian and Poultry Biology Reviews*, *17*(1), 1–20. https://doi.org/10.3184/147020606783437930

Ardia, D. R. (2005). Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. *Journal of Animal Ecology*, *74*(3), 517–524. https://doi.org/10.1111/j.1365-2656.2005.00950.x

Ardia, D. R., Cooper, C. B., & Dhondt, A. A. (2006a). Warm temperatures lead to early onset of incubation, shorter incubation periods and greater hatching asynchrony in tree swallows *Tachycineta bicolor* at the extremes of their range. *Journal of Avian Biology*, *37*(2), 137–142. https://doi.org/10.1111/j.0908-8857.2006.03747.x

Ardia, D. R., Pérez, J. H., Chad, E. K., Voss, M. A., & Clotfelter, E. D. (2009). Temperature and life history: Experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. *Journal of Animal Ecology*, *78*(1), 4–13. https://doi.org/10.1111/j.1365-2656.2008.01453.x

Ardia, D. R., Pérez, J. H., & Clotfelter, E. D. (2006b). Nest box orientation affects internal temperature and nest site selection by tree swallows. *Journal of Field Ornithology*, *77*(3), 339–344. https://doi.org/10.1111/j.1557-9263.2006.00064.x

Ardia, D. R., Pérez, J. H., & Clotfelter, E. D. (2010). Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 1881–1888. https://doi.org/10.1098/rspb.2009.2138

Ardia, D. R., Schat, K. A., & Winkler, D. W. (2003). Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 1679–1683. https://doi.org/10.1098/rspb.2003.2424

Ardia, D. R., Wasson, M. F., & Winkler, D. W. (2006c). Individual quality and food availability determine yolk and egg mass and egg composition in tree swallows *Tachycineta bicolor*. *Journal of Avian Biology*, *37*(3), 252–259. https://doi.org/10.1111/j.2006.0908-8857.03624.x

Arnold, T. W., Rohwer, F. C., & Armstrong, T. (1987). Egg viability, nest predation, and the adaptive significance of clutch size in prairie ducks. *The American Naturalist*, *130*(5), 643–653. https://doi.org/10.1086/284736

Assersohn, K., Marshall, A. F., Morland, F., Brekke, P., & Hemmings, N. (2021). Why do eggs fail? Causes of hatching failure in threatened populations and consequences for conservation. *Animal Conservation*, *24*(4), 540–551. https://doi.org/10.1111/acv.12674

Barber, C. A., Robertson, R. J., & Boag, P. T. (1996). The high frequency of extra-pair paternity in tree swallows is not an artifact of nestboxes. *Behavioral Ecology and Sociobiology*, *38*, 425–430. https://doi.org/10.1007/s002650050260

Bailey, L. D., & van de Pol, M. (2016). climwin: An R toolbox for climate window analysis. *PLoS ONE*, *11*(12), e0167980. https://doi.org/10.1371/journal.pone.0167980

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beck, M. L., & Hopkins, W. A. (2019). The relationship between plumage coloration and aggression in female tree swallows. *Journal of Avian Biology*, *50*(11), 1–11. https://doi.org/10.1111/jav.01910

Beck, M. L., Hopkins, W. A., Jackson, B. P., & Hawley, D. M. (2015). The effects of a remediated fly ash spill and weather conditions on reproductive success and offspring development in tree swallows. *Environmental Monitoring and Assessment*, *187*(3), 119. https://doi.org/10.1007/s10661-015-4333-9

Beissinger, S. R., Cook, M. I., & Arendt, W. J. (2005). The shelf life of bird eggs: Testing egg viability using a tropical climate gradient. *Ecology*, *86*(8), 2164–2175. https://doi.org/10.1890/04-1624

Beltran, R. S., & Tarwater, C. E. (2024). Overcoming the pitfalls of categorizing continuous variables in ecology, evolution and behaviour. *Proceedings of the Royal Society B: Biological Sciences*, *291*(2032), 20241640. https://doi.org/10.1098/rspb.2024.1640

Bentz, A. B., & Siefferman, L. (2013). Age-dependent relationships between coloration and reproduction in a species exhibiting delayed plumage maturation in females. *Journal of Avian Biology*, *44*, 80–88. https://doi.org/10.1111/j.1600-048X.2012.05730.x

Berzins, L. L., & Dawson, R. D. (2016). Experimentally altered plumage brightness of female tree swallows: A test of the differential allocation hypothesis. *Behaviour*, *153*(5), 525–550. https://doi.org/10.1163/1568539X-00003354

Berzins, L. L., & Dawson, R. D. (2020). Does experimentally altered plumage brightness influence extra-pair mating success in female tree swallows (*Tachycineta bicolor*)? *Canadian Journal of Zoology*, *98*(1), 55–61. https://doi.org/10.1139/cjz-2019-0142

Berzins, L. L., Dawson, R. D., Morrissey, C. A., & Clark, R. G. (2020). The relative contribution of individual quality and changing climate as drivers of lifetime reproductive success in a short-lived avian species. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-75557-w

Berzins, L. L., Mazer, A. K., Morrissey, C. A., & Clark, R. G. (2021). Pre-fledging quality and recruitment in an aerial insectivore reflect dynamics of insects, wetlands and climate. *Oecologia*, *196*, 89–100. https://doi.org/10.1007/s00442-021-04918-7

Bidwell, M. T., & Dawson, R. D. (2005). Calcium availability limits reproductive output of tree swallows (*Tachycineta bicolor*) in a nonacidified landscape. *The Auk*, *122*(1), 246–254. https://doi.org/10.1093/auk/122.1.246

Birds Canada, & Environment and Climate Change Canada. (2024). *The state of Canada's birds report*. NatureCounts. https://doi.org/10.71842/8bab-ks08

Birkhead, T. R., Hall, J., Schut, E., & Hemmings, N. (2008). Unhatched eggs: Methods for discriminating between infertility and early embryo mortality. *Ibis*, *150*(3), 508–517. https://doi.org/10.1111/j.1474-919X.2008.00813.x

Birkhead, T. R., Veiga, J. P., & Fletcher, F. (1995). Sperm competition and unhatched eggs in the house sparrow. *Journal of Avian Biology*, *26*(4), 343–345. https://doi.org/10.2307/3677051

Bitton, P.-P., & Dawson, R. D. (2017). Age-related prenatal maternal effects and postnatal breeding experience have different influences on nestling development in an altricial passerine. *Journal of Avian Biology*, *48*(5), 660–668. https://doi.org/10.1111/jav.01202

Bitton, P.-P., Dawson, R. D., & O'Brien, E. L. (2006). Influence of intraclutch egg-mass variation and hatching asynchrony on relative offspring performance within broods of an altricial bird. *Canadian Journal of Zoology*, *84*(12), 1721–1726. https://doi.org/10.1139/z06-179

Bitton, P.-P., Dawson, R. D., & Ochs, C. L. (2008). Plumage characteristics, reproductive investment and assortative mating in tree swallows *Tachycineta bicolor*. *Behavioral Ecology and Sociobiology*, *62*(10), 1543–1550. https://doi.org/10.1007/s00265-008-0583-7

Bitton, P.-P., O'Brien, E. L., & Dawson, R. D. (2007). Plumage brightness and age predict extrapair fertilization success of male tree swallows *Tachycineta bicolor*. *Animal Behaviour*, *74*(6), 1777–1784. https://doi.org/10.1016/j.anbehav.2007.03.018

Bogdanova, M. I., Nager, R. G., & Monaghan, P. (2006). Does parental age affect offspring performance through differences in egg quality? *Functional Ecology*, *20*(1), 132–141. https://www.jstor.org/stable/3598969

Bollinger, P. B., Bollinger, E. K., Daniel, S. L., Gonser, R. A., & Tuttle, E. M. (2018). Partial incubation during egg laying reduces eggshell microbial loads in a temperate-breeding passerine. *Journal of Avian Biology*, *49*(6), Article jav-01560. https://doi.org/10.1111/jav.01560

Both, C., & Visser, M. E. (2005). The effect of climate change on the correlation between avian life-history traits. *Global Change Biology*, *11*, 1606–1613. https://doi.org/10.1111/j.1365-2486.2005.01038.x

Bourret, A., Bélisle, M., Pelletier, F., & Garant, D. (2015). Multidimensional environmental influences on timing of breeding in a tree swallow population facing climate change. *Evolutionary Applications*, 933–944. https://doi.org/10.1111/eva.12315

Briskie, J. V., & Mackintosh, M. (2004). Hatching failure increases with severity of population bottlenecks in birds. *Proceedings of the National Academy of Sciences*, *101*(2), 558–561. https://doi.org/10.1073/pnas.0305103101

Bronson, F. H. (2009). Climate change and seasonal reproduction in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1534), 3331–3340. https://doi.org/10.1098/rstb.2009.0140

Bucevičius, J., Lukinavičius, G., & Gerasimaitė. (2018). The use of Hoechst dyes for DNA staining and beyond. *Chemosensors*, *6*(2), 18. https://doi.org/10.3390/chemosensors6020018

Bussolini, L. T., Crates R., Magrath, M. J. L., & Stojanovic, D. (2023). Identifying factors affecting captive breeding success in a critically endangered species. *Emu - Austral Ornithology*, *123*(2), 161–169. https://doi.org/10.1080/01584197.2023.2194541

Carey, C. (1996). Female reproductive energetics. In *Avian energetics and nutritional ecology* (pp. 324–374). Springer.

Carey, C. (2009). The impacts of climate change on the annual cycles of birds. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1534), 3321–3330. https://doi.org/10.1098/rstb.2009.0182

Challenger, W. O., Williams, T. D., Christians, J. K., & Vézina, F. (2001). Follicular development and plasma yolk precursor dynamics through the laying cycle in the European starling (*Sturnus vulgaris*). *Physiological and Biochemical Zoology*, *74*(3), 356–365. https://doi.org/10.1086/320427

Chen, Q., Di, Z., Roger, E. M. G., Li, H., Richmond, P., & Roehner, B. M. (2020). Magnitude and significance of the peak of early embryonic mortality. *Journal of Biological Physics*, *46*, 233–251. https://doi.org/10.1007/s10867-020-09555-4

Christensen, V. L. (2001). Factors associated with early embryonic mortality. *World's Poultry Science Journal*, *57*(4), 359–372. https://doi.org/10.1079/WPS20010025

Christians, J. K., & Williams, T. D. (2001). Interindividual variation in yolk mass and the rate of growth of ovarian follicles in the zebra finch (*Taeniopygia guttata*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *171*(3), 255–261. https://doi.org/10.1007/s003600100174

Clark, A. B., & Wilson, D. S. (1985). The onset of incubation in birds. *The American Naturalist*, *125*(4), 603–611. https://doi.org/10.1086/284365

Coady, C. D., & Dawson, R. D. (2013). Subadult plumage color of female tree swallows (*Tachycineta bicolor*) reduces conspecific aggression during the breeding season. *The Wilson Journal of Ornithology*, *125*, 348–357. https://doi.org/10.1676/12-155.1

Coe, B. H., Beck, M. L., Chin, S. Y., Jachowski, C. M. B., & Hopkins, W. A. (2015). Local variation in weather conditions influences incubation behavior and temperature in a passerine bird. *Journal of Avian Biology*, *46*(4), 385–394. https://doi.org/10.1111/jav.00581

Conde, D. A., Staerk, J., Colchero, F., da Silva, R., Schöley, J., Baden, H. M., Jouvet, L., Fa, J. E., Syed, H., Jongejans, E., Meiri, S., Gaillard, J. M., Chamberlain, S., Wilcken, J., Jones, O. R., Dahlgren, J. P., Steiner, U. K., Bland, L. M., Gomez-Mestre, I., … Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *PNAS*, *116*(19), 9658–9664. https://doi.org/10.1073/pnas.181636711

Cook, M. I., Beissinger, S. R., Toranzos, G. A., & Arendt, W. J. (2005). Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection: Incubation reduces microbes on eggshells. *Ecology Letters*, *8*(5), 532–537. https://doi.org/10.1111/j.1461-0248.2005.00748.x

Cook, M. I., Beissinger, S. R., Toranzos, G. A., Rodriguez, R. A., & Arendt, W. J. (2003). Trans–shell infection by pathogenic micro–organisms reduces the shelf life of non–incubated bird's eggs: A constraint on the onset of incubation? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 2233–2240. https://doi.org/10.1098/rspb.2003.2508

Cox, A. R., Robertson, R. J., Fedy, B. C., Rendell, W. B., & Bonier, F. (2018). Demographic drivers of local population decline in tree swallows (*Tachycineta bicolor*) in Ontario, Canada. *The Condor*, *120*(4), 842–851. https://doi.org/10.1650/CONDOR-18-42.1

Cox, A. R., Robertson, R. J., Lendvai, Á. Z., Everitt, K., & Bonier, F. (2019). Rainy springs linked to poor nestling growth in a declining avian aerial insectivore (*Tachycineta bicolor*). *Proceedings of the Royal Society B: Biological Sciences*, *286*(1898), 1–8. https://doi.org/10.1098/rspb.2019.0018

Cox, A. R., Robertson, R. J., Rendell, W. B., & Bonier, F. (2020). Population decline in tree swallows (*Tachycineta bicolor*) linked to climate change and inclement weather on the breeding ground. *Oecologia*, *192*, 713–722. https://doi.org/10.1007/s00442-020-04618-8

Cresswell, W., & McCleery, R. (2003). How great tits maintain synchronization of their hatch date with food supply in response to long-term variability in temperature. *Journal of Animal Ecology*, *72*, 356–366. https://doi.org/10.1046/j.1365-2656.2003.00701.x

Croyle, K. E., Durrant, B. S., & Jensen, T. (2015). Detection of oocyte perivitelline membrane-bound sperm: A tool for avian collection management. *Conservation Physiology*, *3*(1), 1–12. https://doi.org/10.1093/conphys/cou060

Cucco, M., Grenna, M., & Pellegrino, I. (2017). Egg characteristics in relation to skipped days of laying in the grey partridge. *Avian Biology Research*, *10*(4). https://doi.org/10.3184/175815617X15036738758853

Custer, C. M., Gray, B. R., & Custer, T. W. (2010). Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, *Tachycineta bicolor*. *Environmental Toxicology and Chemistry*, *29*(4), 909–921. https://doi.org/10.1002/etc.88

De Steven, D. (1978). The influence of age on the breeding biology of the tree swallow *Iridoprocne bicolor*. *Ibis*, *120*, 516–523. https://doi.org/10.1111/j.1474-919X.1978.tb06817.x

Deeming, D. C. (2002). Embryonic development and utilisation of egg components. In D. C. Deeming (Ed.), *Avian incubation: Behaviour, environment and evolution* (pp. 43–53). Oxford University Press.

Deeming, D. C. (2009). The role of egg turning during incubation. *Avian Biology Research*, *2*(1–2), 67–71. https://doi.org/10.3184/175815509X431849

Deeming, D. C., & Ferguson, M. W. J. (1992). Physiological effects of incubation temperature on embryonic development in reptiles and birds. In D. C. Deeming & M. W. J. Ferguson (Eds.), *Egg incubation: Its effects on embryonic development in birds and reptiles* (pp. 147–171). Cambridge University Press.

Di Giovanni, A. J., Miller, M. J., Jones, T. M., Benson, T. J., & Ward, M. P. (2022). Hatching failure is greater in altricial bird species with cavity nests and large clutches. *Ornithology*, *140*, 1–15. https://doi.org/10.1093/ornithology/ukac048

Digby, A., Eason, D., Catalina, A., Lierz, M., Galla, S., Urban, L., Le Lec, M. F., Guhlin, J., Steeves, T. E., Dearden, P. K., Joustra, T., Lees, C., Davis, T., Robertson, B. C., Robertson, F., Vercoe, D., & Kākāpō Recovery Team. (2023). Hidden impacts of conservation management on fertility of the critically endangered kākāpō. *PeerJ*, *11*, e14675. https://doi.org/10.7717/peerj.14675

Dods, P. L., Birmingham, E. M., Williams, T. D., Ikonomou, M. G., Bennie, D. T., & Elliott, J. E. (2005). Reproductive success and contaminants in tree swallows breeding at a wastewater treatment plant. *Environmental Toxicology and Chemistry*, *24*(12), 3106–3112. https://doi.org/10.1897/04-547R.1

Dunn, P. O. (2019). Changes in timing of breeding and reproductive success in birds. In P. O. Dunn & A. P. Møller (Eds.), *Effects of climate change on birds* (2nd ed., pp. 108–119). Oxford University Press.

Dunn, P. O., Ahmed, I., Armstrong, E., Barlow, N., Barnard, M. A., Bélisle, M., Benson, T. J., Berzins, L. L., Boynton, C. K., Brown, T. A., Cady, M., Cameron, K., Chen, X., Clark, R. G., Clotfelter, E. D., Cromwell, K., Dawson, R. D., Denton, E., Forbes, A., … Whittingham, L. A. (2023). Extensive regional variation in the phenology of insects and their response to temperature across North America. *Ecology*, *104*(5), Article e4036. https://doi.org/10.1002/ecy.4036

Dunn, P. O., & Whittingham, L. A. (2007). Search costs influence the spatial distribution, but not the level, of extra-pair mating in tree swallows. *Behavioral Ecology and Sociobiology*, *61*(3), 449–454. https://doi.org/10.1007/s00265-006-0272-3

Dunn, P. O., Winkler, D. W., Whittingham, L. A., Hannon, S. J., & Robertson, R. J. (2011). A test of the mismatch hypothesis: How is timing of reproduction related to food abundance in an aerial insectivore? *Ecology*, *92*(2), 450–461. https://doi.org/10.1890/10-0478.1

Durant, J. M., Hjermann, D. O., Ottersen, G., & Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, *33*, 271–283. https://doi.org/cr2007/33/c033p271

Eeva, T., & Lehikoinen, E. (2010). Polluted environment and cold weather induce laying gaps in great tit and pied flycatcher. *Oecologia*, *162*, 533–539. https://doi.org/10.1007/s00442-009-1468-9

Evans, S. R., & Postma, E. (2024). Counting chicks before they hatch: Extending the observed lifetime to better characterize evolutionary processes in the wild. *Evolution*, Article qpae171. https://doi.org/10.1093/evolut/qpae171

Fasenko, G. M. (2007). Egg storage and the embryo. *Poultry Science*, *86*(5), 1020–1024. https://doi.org/10.1093/ps/86.5.1020

Fernie, K. J., & Letcher, R. J. (2018). Waste-water treatment plants are implicated as an important source of flame retardants in insectivorous tree swallows (*Tachycineta bicolor*). *Chemosphere*, *195*, 29–39. https://doi.org/10.1016/j.chemosphere.2017.12.037

Forstmeier, W., & Ellegren, H. (2010). Trisomy and triploidy are sources of embryo mortality in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1694), 2655–2660. https://doi.org/10.1098/rspb.2010.0394

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage.

Fry, D. M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives*, *103*, 7. https://doi.org/10.1289/ehp.95103s7165

Garrett, D. R., Lamoureux, S., Paquette, S. R., Pelletier, F., Garant, D., & Bélisle, M. (2022). Combined effects of cold snaps and agriculture on the growth rates of tree swallows (*Tachycineta bicolor*). *Canadian Journal of Zoology*, *100*(10), 630–646. https://doi.org/10.1139/cjz-2021-0210

Garrett, D. R., Pelletier, F., Garant, D., & Bélisle, M. (2022). Interacting effects of cold snaps, rain, and agriculture on the fledging success of a declining aerial insectivore. *Ecological Applications*, *32*, 1–16. https://doi.org/10.1002/eap.2645

Ghilain, A., & Bélisle, M. (2008). Breeding success of tree swallows along a gradient of agricultural intensification. *Ecological Applications*, *18*(5), 1140–1154. https://doi.org/10.1890/07-1107.1

Godwin, C. M., Barclay, R. M. R., & Smits, J. E. G. (2019). Tree swallow (*Tachycineta bicolor*) nest success and nestling growth near oil sands mining operations in northeastern Alberta, Canada. *Canadian Journal of Zoology*, *97*, 547–557. https://doi.org/10.1139/cjz-2018-0247

Gonzalez, A., Satterlee, D. G., Moharer, F., & Cadd, G. G. (1999). Factors affecting ostrich egg hatchability. *Poultry Science*, *78*(9), 1257–1262. https://doi.org/10.1093/ps/78.9.1257

Government of Canada. (2025, February 17). *Station results*. Historical Data. https://climate.weather.gc.ca/historical_data/search_historic_data_stations_e.html?searchType=stnName&timeframe=1&txtStationName=prince+george&searchMethod=contains&optLimit=yearRange&StartYear=1840&EndYear=2025&Year=2025&Month=2&Day=16&selRowPerPage=25

Grafen, A. (1988). On the uses of data on lifetime reproductive success. In T. H. Clutton-Brock (Ed.), *Reproductive Success* (pp. 454–471). University of Chicago Press.

Griebel, I. A., & Dawson, R. D. (2019). Predictors of nestling survival during harsh weather events in an aerial insectivore, the tree swallow (*Tachycineta bicolor*). *Canadian Journal of Zoology*, *97*(2), 81–90. https://doi.org/10.1139/cjz-2018-0070

Grüebler, M., Morand, M., & Naefdaenzer, B. (2008). A predictive model of the density of airborne insects in agricultural environments. *Agriculture, Ecosystems & Environment*, *123*(1–3), 75–80. https://doi.org/10.1016/j.agee.2007.05.001

Guraya, S. S. (1989). Follicular atresia. In *Ovarian follicles in reptiles and birds* (Vol. 24). Springer.

Hahn, D. C. (2011). Patterns of maternal yolk hormones in eastern screech owl eggs (Megascops asio). *General and Comparative Endocrinology*, *172*(3), 423–429. https://doi.org/10.1016/j.ygcen.2011.04.001

Hamburger, V., & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Developmental Dynamics*, *195*(4), 231–272. https://doi.org/10.1002/aja.1001950404

Harriman, V. B., Dawson, R. D., Bortolotti, L. E., & Clark, R. G. (2017). Seasonal patterns in reproductive success of temperate-breeding birds: Experimental tests of the date and quality hypotheses. *Ecology and Evolution*, *7*(7), 2122–2132. https://doi.org/10.1002/ece3.2815

Harriman, V. B., Dawson, R. D., Clark, R. G., Fairhurst, G. D., & Bortolotti, G. R. (2014). Effects of ectoparasites on seasonal variation in quality of nestling tree swallows (*Tachycineta bicolor*). *Canadian Journal of Zoology*, *92*, 87–96. https://doi.org/10.1139/cjz-2013-0209

Harris, M. E. (2023). Foraging habitat use and diet composition of barn swallows (*Hirundo rustica*) and tree swallows (*Tachycineta bicolor*) in agriculturally intensive landscapes of the northern prairies [Master's Thesis, University of Saskatchewan]. HARVEST. https://hdl.handle.net/10388/14450

Harris, M. E., Hobson, K. A., & Morrissey, C. A. (2024). *Hirundo rustica* (barn swallows) and *Tachycineta bicolor* (tree swallows) select wetlands in agriculturally intensive landscapes, as revealed by GPS tracking. *Ornithological Applications*, *126*(3), duae012. https://doi.org/10.1093/ornithapp/duae012

Hartig, F. (2024). *Residual diagnostics for hierarchical (multi-level / mixed) regression models* (Version 0.4.7) [Computer software]. https://CRAN.R-project.org/package=DHARMa

Hasselquist, D., Wasson, M. F., & Winkler, D. W. (2001). Humoral immunocompetence correlates with date of egg-laying and reflects work load in female tree swallows.

Behavioral Ecology, *12*(1), 93–97. https://doi.org/10.1093/oxfordjournals.beheco.a000384

Hemmings, N., & Birkhead, T. R. (2015). Polyspermy in birds: Sperm numbers and embryo survival. *Proceedings of the Royal Society B: Biological Sciences*, *282*, Article 20151682. https://doi.org/10.1098/rspb.2015.1682

Hemmings, N., & Birkhead, T. R. (2016). Consistency of passerine embryo development and the use of embryonic staging in studies of hatching failure. *Ibis*, *158*(1), 43–50. https://doi.org/10.1111/ibi.12336

Hemmings, N., & Evans, S. (2020). Unhatched eggs represent the invisible fraction in two wild bird populations. *Biology Letters*, *16*, Article 20190763. https://doi.org/10.1098/rsbl.2019.0763

Hemmings, N., West, M., & Birkhead, T. R. (2012). Causes of hatching failure in endangered birds. *Biology Letters*, *8*(6), 964–967. https://doi.org/10.1098/rsbl.2012.0655

Henderson, L. J., Evans, N. P., Heidinger, B. J., Adams, A., & Arnold, K. E. (2014). Maternal condition but not corticosterone is linked to offspring sex ratio in a passerine bird. *PLoS ONE*, *9*(12), Article e116366. https://doi.org/10.1371/journal.pone.0110858

Hussell, D. J. T. (1983). Age and plumage color in female tree swallows. *Journal of Field Ornithology*, *54*(3), 312–318.

Hussell, D. J. T., & Quinney, T. E. (2008). Food abundance and clutch size of tree swallows *Tachycineta bicolor*. *Ibis*, *129*, 243–258. https://doi.org/10.1111/j.1474-919X.1987.tb03204.x

Jenouvrier, S. (2013). Impacts of climate change on avian populations. *Global Change Biology*, *19*, 2036–2057. https://doi.org/10.1111/gcb.12195

Johnson, C. G. (1950). The comparison of suction trap, sticky trap and tow-net for the quantitative sampling of small airborne insects. *Annals of Applied Biology*, *37*(2), 268–285. https://doi.org/10.1111/j.1744-7348.1950.tb01045.x

Johnson, L. S., Napolillo, F. M., Kozlovsky, D. Y., Hebert, R. M., & Allen, A. (2013). Variation in incubation effort during egg laying in the mountain bluebird and its association with hatching asynchrony. *Journal of Field Ornithology*, *84*(3), 242–254. https://doi.org/10.1111/jofo.12023

Johnston, S. A., & Gous, R. M. (2003). An improved mathematical model of the ovulatory cycle of the laying hen. *British Poultry Science*, *44*(5), 752–760. https://doi.org/10.1080/00071660310001643723

Jones, J. (2003). Tree swallows (*Tachycineta bicolor*): A new model organism? *The Auk*, *120*(3), 591–599. https://doi.org/10.1093/auk/120.3.591

Jönsson, K. I. (1997). Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, *78*(1), 57–66. https://doi.org/10.2307/3545800

Joyner, C. J., Peddie, M. J., & Taylor, T. G. (1987). The effect of age on egg production in the domestic hen. *General and Comparative Endocrinology*, *65*(3), 331–336. https://doi.org/10.1016/0016-6480(87)90117-1

Kato, T., Matsui, S., Terai, Y., Tanabe, H., Hashima, S., Kasahara, S., Morimoto, G., Mikami, O. K., Ueda, K., & Kutsukake, N. (2017). Male-specific mortality biases secondary sex ratio in Eurasian tree sparrows *Passer montanus*. *Ecology and Evolution*, *7*, 10675–10682. https://doi.org/10.1002/ece3.3575

Kempenaers, B., Congon, B., Boag, P., & Robertson, R. (1999). Extrapair paternity and egg hatchability in tree swallows: Evidence for the genetic compatibility hypothesis? *Behavioral Ecology*, *10*(3), 304–311. https://doi.org/10.1093/beheco/10.3.304

Kennamer, R. A., Alsum, S. K., & Colwell, S. V. (1997). Composition of wood duck eggs in relation to egg size, laying sequence, and skipped days of laying. *The Auk*, *114*(3), 479–487. https://doi.org/10.2307/4089248

Kirikçi, K., Deeming, D. C., & Gunlu, A. (2004). Effects of egg mass and percentage mass loss during incubation on hatchability of eggs of the rock partridge (*Alectoris graeca*). *British Poultry Science*, *45*(3), 380–384. https://doi.org/10.1080/00071660410001730879

Koenig, W. D. (1982). Ecological and social factors affecting hatchability of eggs. *The Auk*, *99*(3), 526–536. https://doi.org/10.1093/auk/99.3.526

Krist, M. (2011). Egg size and offspring quality: A meta-analysis in birds. *Biological Reviews*, *86*(3), 692–716. https://doi.org/10.1111/j.1469-185X.2010.00166.x

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lees, A. C., Haskell, L., Allinson, T., Bezeng, S. B., Burfield, I. J., Renjifo, L. M., Rosenberg, K. V., Viswanathan, A., & Butchart, S. H. M. (2022). State of the world's birds. *Annual Review of Environment and Resources*, *47*, 231–260. https://doi.org/10.1146/annurev-environ-112420-014642

Leffelaar, D., & Robertson, R. J. (1985). Nest usurpation and female competition for breeding opportunities by tree swallows. *The Wilson Bulletin*, *97*(2), 221–224.

Lessells, C. M., Dingemanse, N. J., & Both, C. (2002). Egg weights, egg component weights, and laying gaps in great tits (*Parus major*) in relation to ambient temperature. *The Auk*, *119*(4), 1091–1103. https://doi.org/10.1093/auk/119.4.1091

Lifjeld, J. T., Dunn, P. O., & Whittingham, L. A. (2002). Short-term fluctuations in cellular immunity of tree swallows feeding nestlings. *Oecologia*, *130*, 185–190. https://doi.org/10.1007/s004420100798

Liptói, K., & Hidas, A. (2006). Investigation of possible genetic background of early embryonic mortality in poultry. *World's Poultry Science Journal*, *62*(2), 326–337. https://doi.org/10.1079/WPS2005101

Lombardo, M. P. (1994). Nest architecture and reproductive performance in tree swallows (*Tachycineta bicolor*). *The Auk*, *111*(4), 814–824. https://doi.org/10.2307/4088813

Lombardo, M. P. (1995). Effect of feathers as nest insulation on incubation behavior and reproductive performance of tree swallows (*Tachycineta bicolor*). *The Auk*, *112*(4), 973–981. https://doi.org/10.2307/4089028

Lombardo, M. P., & Thorpe, P. A. (2010). Local breeding experience and the reproductive performance of tree swallows: Experience and reproduction in tree swallows. *Journal of Field Ornithology*, *81*(3), 294–301. https://doi.org/10.1111/j.1557-9263.2010.00285.x

Lombardo, M. P., Thorpe, P. A., Otieno, S., Weglarz, D., & Hawker, A. (2021). Factors associated with the mass of eggs laid by tree swallows *Tachycineta bicolor* varied yearly between 2008 and 2016 in Michigan. *Journal of Avian Biology*, *52*(1), 1–12. https://doi.org/10.1111/jav.02460

Macaulay, E. D. M., Tatchell, G. M., & Taylor, L. R. (1988). The Rothamsted insect survey "12-metre" suction trap. *Bulletin of Entomological Research*, *78*(1), 121–128. https://doi.org/10.1017/S0007485300016126

Magrath, R. D. (1992). The effect of egg mass on the growth and survival of blackbirds: A field experiment. *Journal of Zoology*, *227*(4), 639–654. https://doi.org/10.1111/j.1469-7998.1992.tb04420.x

Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2019). pavo 2: New tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution*, *10*(7). https://doi.org/10.1111/2041-210X.13174

Maibach, E., Myers, T., & Leiserowitz, A. (2014). Climate scientists need to set the record straight: There is a scientific consensus that human-caused climate change is happening. *Earth's Future*, *2*(5), 295–298. https://doi.org/10.1002/2013EF000226

Marshall, A. F., Balloux, F., Hemmings, N., & Brekke, P. (2023). Systematic review of avian hatching failure and implications for conservation. *Biological Reviews*, *98*(3), 807–832. https://doi.org/10.1111/brv.12931

Martin, T. E., Tobalske, B., Riordan, M. M., Case, S. B., & Dial, K. P. (2018). Age and performance at fledging are a cause and consequence of juvenile mortality between life stages. *Science Advances*, *4*(6), 1–8. https://doi.org/10.1126/sciadv.aar1988

McCarty, J. P., & Winkler, D. W. (1999). Foraging ecology and diet selectivity of tree swallows feeding nestlings. *The Condor*, *101*(2), 246–254. https://doi.org/10.2307/1369987

Meidinger, D., Pojar, J., & Harper, W. L. (1991). Chapter 14: Sub-Boreal Spruce Zone. In D. Meidinger & J. Pojar (Eds.), *Ecosystems of British Columbia* (pp. 209–221). B.C. Ministry of Forests and Range Research Branch.

Monrós, J. S., Belda, E. J., & Barba, E. (1998). Delays of the hatching dates in great tits *Parus major*: Effects on breeding performance. *Ardea*, *86*, 213–220.

Morland, F., Ewen, J. G., Santure, A. W., Brekke, P., & Hemmings, N. (2024). Demographic drivers of reproductive failure in a threatened bird: Insights from a decade of data. *PNAS*, *121*(36), Article e2319104121. https://doi.org/10.1073/pnas.2319104121

Murray, J. R., Varian-Ramos, C. W., Welch, Z. S., & Saha, M. S. (2013). Embryological staging of the zebra finch, *Taeniopygia guttata*. *Journal of Morphology*, *274*(10), 1090–1110. https://doi.org/10.1002/jmor.20165

Narushin, V. G., & Romanov, M. N. (2002). Egg physical characteristics and hatchability. *World's Poultry Science Journal*, *58*(3), 297–303. https://doi.org/10.1079/WPS20020023

Nebel, S., Mills, A., McCracken, J. D., & Taylor, P. D. (2010). Declines of aerial insectivores in North America follow a geographic gradient. *Avian Conservation and Ecology*, *5*(2), 1–14. https://doi.org/10.5751/ACE-00391-050201

Nilsson, J.-A., & Svensson, E. (1993). The frequency and timing of laying gaps. *Ornis Scandinavica*, *24*(2), 122–126. https://doi.org/10.2307/3676361

Nooker, J. K., Dunn, P. O., & Whittingham, L. A. (2005). Effects of food abundance, weather, and female condition on reproduction in tree swallows. *The Auk*, *122*(4), 1225–1238. https://doi.org/10.1093/auk/122.4.1225

Nord, A., & Williams, J. B. (2015). The energetic costs of incubation. In D. C. Deeming & S. J. Reynolds (Eds.), *Nests, Eggs, and Incubation* (pp. 152–170). Oxford University Press.

Ojanen, M. (1983). Egg development and the related nutrient reserve depletion in the pied flycatcher, Ficedula hypoleuca. *Annales Zoologici Fennici*, *20*(4), 293–299.

Pellerin, S., Rioux Paquette, S., Pelletier, F., Garant, D., & Bélisle, M. (2016). The trade-off between clutch size and egg mass in tree swallows *Tachycineta bicolor* is modulated by female body mass. *Journal of Avian Biology*, *47*, 500–507.

Perrins, C. M. (1996). Eggs, egg formation and the timing of breeding. *Ibis*, *138*(1), 2–15. https://doi.org/10.1111/j.1474-919X.1996.tb04308.x

Prum, R. O. (2006). Anatomy, physics, and evolution of structural colors. In G. E. Hill & K. J. McGraw (Eds.), *Bird coloration volume I: Mechanisms and measurements* (pp. 295–353). Harvard University Press.

Quinney, T. E., & Ankney, C. D. (1985). Prey size selection by tree swallows. *The Auk*, *102*(2), 245–250. https://doi.org/10.2307/4086766

Quinney, T. E., Hussell, D. J. T., & Ankney, C. D. (1986). Sources of Variation in Growth of Tree Swallows. *The Auk*, *103*(2), 389–400. https://doi.org/10.1093/auk/103.2.389

R Core Team. (2023). *R: A language and environment for statistical computing* [Computer software]. R Foundation for Statistical Computing. https://www.R-project.org/

Ramachandran, R., & McDaniel, C. D. (2018). Parthenogenesis in birds: A review. *Reproduction*, *155*(6), R245–R257. https://doi.org/10.1530/REP-17-0728

Remeš, V. (2007). Avian growth and development rates and age-specific mortality: The roles of nest predation and adult mortality. *Journal of Evolutionary Biology*, *20*(1), 320–325. https://doi.org/10.1111/j.1420-9101.2006.01191.x

Remeš, V. (2011). Yolk androgens in great tit eggs are related to male attractiveness, breeding density and territory quality. *Behavioral Ecology and Sociobiology*, *65*, 1257–1266. https://doi.org/10.1007/s00265-011-1139-9

Romanoff, A. L. (1972). *Pathogenesis of the avian embryo: Causes of malformations and prenatal death* (1st ed.). Wiley-Interscience.

Rooneem, T. M., & Robertson, R. J. (1997). The potential to lay replacement clutches by tree swallows. *The Condor*, *99*(1), 228–231. https://doi.org/10.2307/1370246

Rosenberg, K. V., Dokter, A. M., Blancher, P. J., Sauer, J. R., Smith, A. C., Smith, P. A., Stanton, J. C., Panjabi, A., Helft, L., Parr, M., & Marra, P. P. (2019). Decline of the North American avifauna. *Science*, *366*, 120–124. https://doi.org/10.1126/science.aaw1313

Rotenberry, J. T., & Balasubramaniam, P. (2020). Estimating egg mass-body mass relationships in birds. *The Auk*, *137*, 1–11. https://doi.org/10.1093/auk/ukaa019

Ryder, O. A., Thomas, S., Judson, J. M., Romanov, M. N., Dandekar, S., Papp, J. C., Sidak-Loftis, L. C., Walker, K., Stalis, I. H., Mace, M., Steiner, C. C., & Chemnick, L. G. (2021). Facultative parthenogenesis in California condors. *Journal of Heredity*, *112*(7), 569–574. https://doi.org/10.1093/jhered/esab052

Sánchez-Lafuente, A. M. (2004). Trade-off between clutch size and egg mass, and their effects on hatchability and chick mass in semi-precocial purple swamphen. *Ardeola*, *51*(2), 319–330.

Schut, E., Hemmings, N., & Birkhead, T. R. (2008). Parthenogenesis in a passerine bird, the zebra finch *Taeniopygia guttata*. *Ibis*, *150*, 197–199. https://doi.org/10.1111/j.1474-919X.2007.00755.x

Shawkey, M. D., Firestone, M. K., Brodie, E. L., & Beissinger, S. R. (2009). Avian incubation inhibits growth and diversification of bacterial assemblages on eggs. *PLoS ONE*, *4*, Article e4522. https://doi.org/4510.1371/journal.pone.0004522

Shipley, J. R., Twining, C. W., Taff, C. C., Vitousek, M. N., Flack, A., & Winkler, D. W. (2020). Birds advancing lay dates with warming springs face greater risk of chick mortality. *PNAS*, *117*(41), 25590–25594. https://doi.org/10.1073/pnas.2009864117

Shook, J. G., Stephenson, A. B., & Biellier, H. V. (1971). Heritability estimates of differences in arbitrary embryonic mortality traits in turkeys. *Poultry Science*, *50*(5), 1255–1260. https://doi.org/10.3382/ps.0501255

Shutler, D., Clark, R. G., Fehr, C., & Diamond, A. W. (2006). Time and recruitment costs as currencies in manipulation studies on the costs of reproduction. *Ecology*, *87*(11), 2938–2946. https://doi.org/10.1890/0012-9658(2006)87[2938:TARCAC]2.0.CO;2

Small, A. O., Schlusser, K., Ryan, C. J., & Jamieson, I. G. (2000). Detecting sperm on the perivitelline membrane of incubated turkey eggs and its implications for research on fertility problems in endangered species. *Wildlife Research*, *27*(6), 635–637. https://doi.org/10.1071/WR99080

Smith, A. C., Hudson, M.-A. R., Downes, C. M., & Francis, C. M. (2015). Change points in the population trends of aerial-insectivorous birds in North America: Synchronized in time across species and regions. *PLoS ONE*, *10*(7), 1–23. https://doi.org/10.1371/journal.pone.0130768

Sockman, K. W. (2008). Ovulation order mediates a trade-off between pre-hatching and post-hatching viability in an altricial bird. *PLoS ONE*, *3*(3), Article e1785. https://doi.org/10.1371/journal.pone.0001785

Soma, M., Saito, D. S., Hasegawa, T., & Okanoya, K. (2007). Sex-specific maternal effect on egg mass, laying order, and sibling competition in teh Bengalese finch (*Lonchura striata* var. *domestica*). *Behavioral Ecology and Sociobiology*, *61*, 1695–1705. https://doi.org/10.1007/s00265-007-0400-8

Somveille, M., Rodrigues, A. S. L., & Manica, A. (2018). Energy efficiency drives the global seasonal distribution of birds. *Nature Ecology & Evolution*, *2*, 962–969. https://doi.org/10.1038/s41559-018-0556-9

Spiller, K. J., & Dettmers, R. (2019). Evidence for multiple drivers of aerial insectivore declines in North America. *The Condor*, *121*(2), 1–13. https://doi.org/10.1093/condor/duz010

Stutchbury, B. J., & Robertson, R. J. (1988). Within-season and age-related patterns of reproductive performance in female tree swallows (*Tachycineta bicolor*). *Canadian Journal of Zoology*, *66*(4), 827–834. https://doi.org/10.1139/z88-122

Taff, C. C., Ryan, T. A., Uehling, J. J., Injaian, A. S., & Vitousek, M. N. (2022). Within-individual consistency and between-individual variation in the shapes of eggs laid by tree swallows (*Tachycineta bicolor*). bioRxiv. https://doi.org/10.1101/2022.02.02.478835

Taylor, C. E., & Cristol, D. A. (2015). Tissue mercury concentrations and survival of tree swallow embryos, nestlings and young adult females on a contaminated site. *Bulletin of Environmental Contamination and Toxicology*, *95*(4), 459–464. https://doi.org/10.1007/s00128-015-1643-0

Taylor, L. R. (1963). Analysis of the effect of temperature on insects in flight. *Journal of Animal Ecology*, *32*(1), 99–117. https://doi.org/10.2307/2520

Tazawa, H., & Whittow, G. C. (2000). Incubation physiology. In P. D. Sturkie (Ed.), *Avian physiology* (5th ed., pp. 617–634). Academic Press.

Tomás, G. (2015). Hatching date vs laying date: What should we look at to study avian optimal timing of reproduction? *Journal of Avian Biology*, *46*(1), 107–112. https://doi.org/10.1111/jav.00499

Twining, C. W., Brenna, J. T., Shipley, J. R., Tollefson, T. N., & Winkler, D. W. (2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. *PNAS*, *113*(39), 10920–10925. https://doi.org/10.1073/pnas.1603998113

Twining, C. W., Shipley, J. R., & Winkler, D. W. (2018). Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecology Letters*, *21*(12), 1812–1820. https://doi.org/10.1111/ele.13156

van de Pol, M., Bailey, L. D., McLean, N., Rijsdijk, L., Lawson, C. R., & Brouwer, L. (2016). Identifying the best climatic predictors in ecology and evolution. *Methods in Ecology and Evolution*, *7*(10), 1246–1257. https://doi.org/10.1111/2041-210X.12590

Vázquez, D. P., Gianoli, E., Morris, W. F., & Bozinovic, F. (2017). Ecological and evolutionary impacts of changing climatic variability. *Biological Reviews*, *92*(1), 22–42. https://doi.org/10.1111/brv.12216

Vedder, O. (2012). Individual birds advance offspring hatching in response to increased temperature after the start of laying. *Oecologia*, *170*, 619–628. https://doi.org/10.1007/s00442-012-2335-7

Viñuela, J. (2000). Opposing selective pressures on hatching asynchrony: Egg viability, brood reduction, and nestling growth. *Behavioral Ecology and Sociobiology*, *48*(5), 333–343. https://doi.org/10.1007/s002650000245

Wang, J. M., & Beissinger, S. R. (2009). Variation in the onset of incubation and its influence on avian hatching success and asynchrony. *Animal Behaviour*, *78*(3), 601–613. https://doi.org/10.1016/j.anbehav.2009.05.022

Wang, J. M., & Beissinger, S. R. (2011). Partial incubation in birds: Its occurrence, function, and quantification. *The Auk*, *128*(3), 454–466. https://doi.org/10.1525/auk.2011.10208

Wardrop, S. L., & Ydenberg, R. C. (2003). Date and parental quality effects in the seasonal decline in reproductive performance of the tree swallow *Tachycineta bicolor*: Interpreting results in light of potential experimental bias. *Ibis*, *145*, 439–447. https://doi.org/10.1046/j.1474-919X.2003.00184.x

Watt, J. M., Petitte, J. N., & Etches, R. J. (1993). Early development of the chick embryo. *Journal of Morphology*, *215*, 165–182. https://doi.org/10.1002/jmor.1052150205

Webb, D. R. (1987). Thermal tolerance of avian embryos: A review. *The Condor*, *89*(4), 874–898. https://doi.org/10.2307/1368537

Whittingham, L. A., & Dunn, P. O. (2016). Experimental evidence that brighter males sire more extra-pair young in tree swallows. *Molecular Ecology*, *25*(15), 3706–3715. https://doi.org/10.1111/mec.13665

Whittingham, L. A., Dunn, P. O., & Lifjeld, J. T. (2007). Egg mass influences nestling quality in tree swallows, but there is no differential allocation in relation to laying order or sex. *Ornithological Applications*, *109*(3), 585–594. https://doi.org/10.1093/condor/109.3.585

Wiebe, K. L., & Martin, K. (1995). Ecological and physiological effects on egg laying intervals in ptarmigan. *The Condor*, *97*(3), 708–717. https://doi.org/10.2307/1369179

Williams, T. D. (2005). Mechanisms underlying the costs of egg production. *BioScience*, *55*(1), 39–48. https://doi.org/10.1641/0006-3568(2005)055[0039:MUTCOE]2.0.CO;2

Williams, T. D. (2012). *Physiological Adaptations for Breeding in Birds*. Princeton University Press.

Wilson, H. R. (1990). Physiological requirements of the developing embryo: Temperature and turning. *Avian Incubation*, 145–156. https://doi.org/10.5555/19910188960

Winkler, D. W., & Allen, P. E. (1995). Effects of handicapping on female condition and reproduction in tree swallows. *The Auk*, *112*(3), 737–747. https://doi.org/10.1093/auk/112.3.737

Winkler, D. W., & Allen, P. E. (1996). The seasonal decline in tree swallow clutch size: Physiological constraint or strategic adjustment? *Ecology*, *77*(3), 922–932. https://doi.org/10.2307/2265512

Winkler, D. W., Hallinger, K. K., Ardia, D. R., Robertson, R. J., Stutchbury, B. J., & Cohen, R. R. (2020a). Tree Swallow (*Tachycineta bicolor*), version 1.0. In A. F. Poole (Ed.), *Birds of the World*. https://doi.org/10.2173/bow.treswa.01

Winkler, D. W., Hallinger, K. K., Pegan, T. M., Taff, C. C., Verhoeven, M. A., Chang van Oordt, D., Stager, M., Uehling, J. J., Vitousek, M. N., Andersen, M. J., Ardia, D. R., Belmaker, A., Ferretti, V., Forsman, A. M., Gaul, J. R., Llambias, P. E., Orzechowski, S. C., Shipley, J. R., Wilson, M., & Yoon, H. S. (2020b). Full lifetime perspectives on the costs and benefits of lay-date variation in tree swallows. *Ecological Society of America*, *101*(9), 1–19. https://doi.org/10.1002/ecy.3109

Winkler, D. W., Luo, M. K., & Rakhimberdiev, E. (2013). Temperature effects on food supply and chick mortality in tree swallows (*Tachycineta bicolor*). *Oecologia*, *173*(1), 129–138. https://doi.org/10.1007/s00442-013-2605-z

Zach, R. (1982). Hatching asynchrony, egg size, growth, and fledging in tree swallows. *The Auk*, *99*, 695–700. https://doi.org/10.1093/auk/99.4.695

Appendix A: Aerial insect sampling

A.1 INTRODUCTION

The diet of breeding female tree swallows (*Tachycineta bicolor*) during the period of rapid yolk deposition contributes to the quantity and quality of nutrients provided to developing embryos (Ardia et al., 2006c; Jönsson, 1997; Williams, 2012). I used passive aerial insect samplers to measure the prey available to foraging tree swallows during the egg laying period with the intent of testing the relationship between prey availability and embryonic viability in Chapter 3. I expected the daily biomass of insects sampled at each site to be related to the daily maximum temperature and positively correlated with egg mass, as has been demonstrated in other research on tree swallows (e.g., Ardia et al., 2006c; Garrett et al., 2022); however, the data I collected were inconsistent with these predictions and many other studies. Due to these inconsistencies, I did not use insect biomass as a predictor variable in my models.

A.2 METHODS

I measured prey abundance during the egg-laying period using samplers designed to capture aerial insects in passive tow nets at each study site in Chapter 3 (design described by Quinney & Ankney, 1985). Four samplers were deployed across the agricultural site, and three samplers were distributed around the wetland site. Each sampler was set at a height of approximately 2 m and designed to immediately euthanize insects in a collection jar filled with 70% ethanol. Jars were replaced daily in 2022 and every second day in 2023, and I stored collected insects in 90% ethanol at room temperature until further processing.

Additionally, I measured local ambient temperature (ºC) and wind speed (km/hr) using weather stations deployed to each site.

Individual insects with a body size greater than 10 mm were removed from each sample prior to drying because tree swallows usually do not forage for insects larger than 10 mm (Quinney & Ankney, 1985). Additionally, I removed wingless ants and adult spiders that likely climbed into the tow net, as well as all plant matter, from each sample. The samples were dried at 70 ºC in an oven for at least eight hours, then immediately weighed using an analytical balance (nearest 0.0001 g). Using the time at which each sample was collected, I determined the mass of insects collected from all samplers at each site per day. To account for the difference in the number of samplers at each site, I multiplied the daily dry masses at the wetland site by 1.33. I then applied a correction for wind speed as described in Quinney et al. (1986) to obtain a final daily insect index per site (mg dry weight/km wind). While insects were not systematically identified, I made general observations of the families and orders present in the samples.

All statistical analyses were conducted in R version 4.3.1 (R Development Core Team, 2023). I used Student's t-tests to compare differences in daily maximum temperature, mean wind speed, and insect biomass between study sites within each year, as well as to compare insect biomass between 2022 and 2023. Data from 13 May – 08 Jun were used in all t-tests because these dates were within the egg formation periods at both study sites during both years. While Garrett et al. (2022) demonstrated that the abundance of dipteran insects is quadratically related to daily maximum temperature, the relationship between aerial insect abundance and temperature may also be linear or non-linear depending on the taxa, habitat type, and geographic location (Dunn et al., 2023; Grüebler et al., 2008; Winkler et al., 2013). I therefore tested both the linear and quadratic relationships between the daily insect index and daily maximum temperature for both sites in 2022 and 2023 using generalized linear mixed effects models fit using the “lme4” package (Bates et al., 2015), with P-values generated by the “lmerTest” package (Kuznetsova et al., 2017). Because the daily maximum temperature two days before an egg is laid is closely related to the mass of that egg (Chapter 3), I also tested the relationship between egg mass and insect biomass two days before egg laying in each year. Study site and female identity were included as nested random effects in all models. Significance was set at alpha = 0.05, and all regression coefficients (β) and means are reported ± 1 standard error.

A.3 RESULTS

During the egg laying period, mean daily insect biomass was significantly greater in 2022 (22.20 ± 5.64 mg/km wind) than 2023 (3.07 ± 0.82 mg/km wind; $t = 3.16$, $df = 26$, $P = 0.004$; Figure A.1), and was significantly greater at the agricultural site than the wetland site in both 2022 and 2023 (Table A.1). Peak insect activity at the agricultural site in both years was associated with a high abundance of March flies (*Bibionidae*; order Diptera, suborder Nematocera) that was not observed at the wetland site (pers. obs.). Ambient temperature was comparable at both sites and the agricultural site was more windy than the wetland site (Table A.1).

Egg mass was not predicted by the insect index two days before each egg was laid in 2022 ($\beta = 0.007 \pm 0.028$, $t = 0.25$, $P = 0.80$) or 2023 ($\beta = -0.28 \pm 0.38$, $t = -0.74$, $P = 0.46$). The quadratic function between daily insect abundance and maximum temperature was significant in 2022 ($\beta = 0.67 \pm 0.17$, $t = 3.94$, $P < 0.001$) and not significant in 2023 (Figure A.2A). The linear function between daily insect biomass and maximum temperature was positively related in both 2022 (β = 0.65 ± 0.11, t = 5.99, $P < 0.001$) and 2023 (β = 0.04 ± 0.01, t = 6.14, $P < 0.001$; Figure A.2B).

A.3.1 Tables

Table A.1. Daily mean ± SE and Student's t-test results for maximum temperature, wind speed, and insect biomass (calculated as an index of mg dry weight/km wind) at an agricultural site and a wetland site near Prince George, British Columbia, Canada. Results are for the egg formation period (13 May – 08 Jun) of breeding tree swallows (*Tachycineta bicolor*) in this region. Bolded P-values are considered significant at alpha = 0.05.

	2022					2023				
	Agricultural	Wetland	t	df	P	Agricultural	Wetland	t	df	P
Temperature (ºC)	16.05 ± 0.74	17.45 ± 0.78	1.30	52	0.20	22.06 ± 1.09	23.45 ± 1.13	0.88	52	0.38
Wind (km/hr)	1.72 ± 0.10	1.26 ± 0.09	-3.44	52	**0.001**	1.45 ± 0.12	0.99 ± 0.09	-2.96	52	**0.005**
Insect index (mg/km wind)	16.10 ± 4.57	5.36 ± 1.45	2.26	52	**0.03**	2.91 ± 0.81	0.15 ± 0.03	3.41	52	**0.001**

A.3.2 Figures

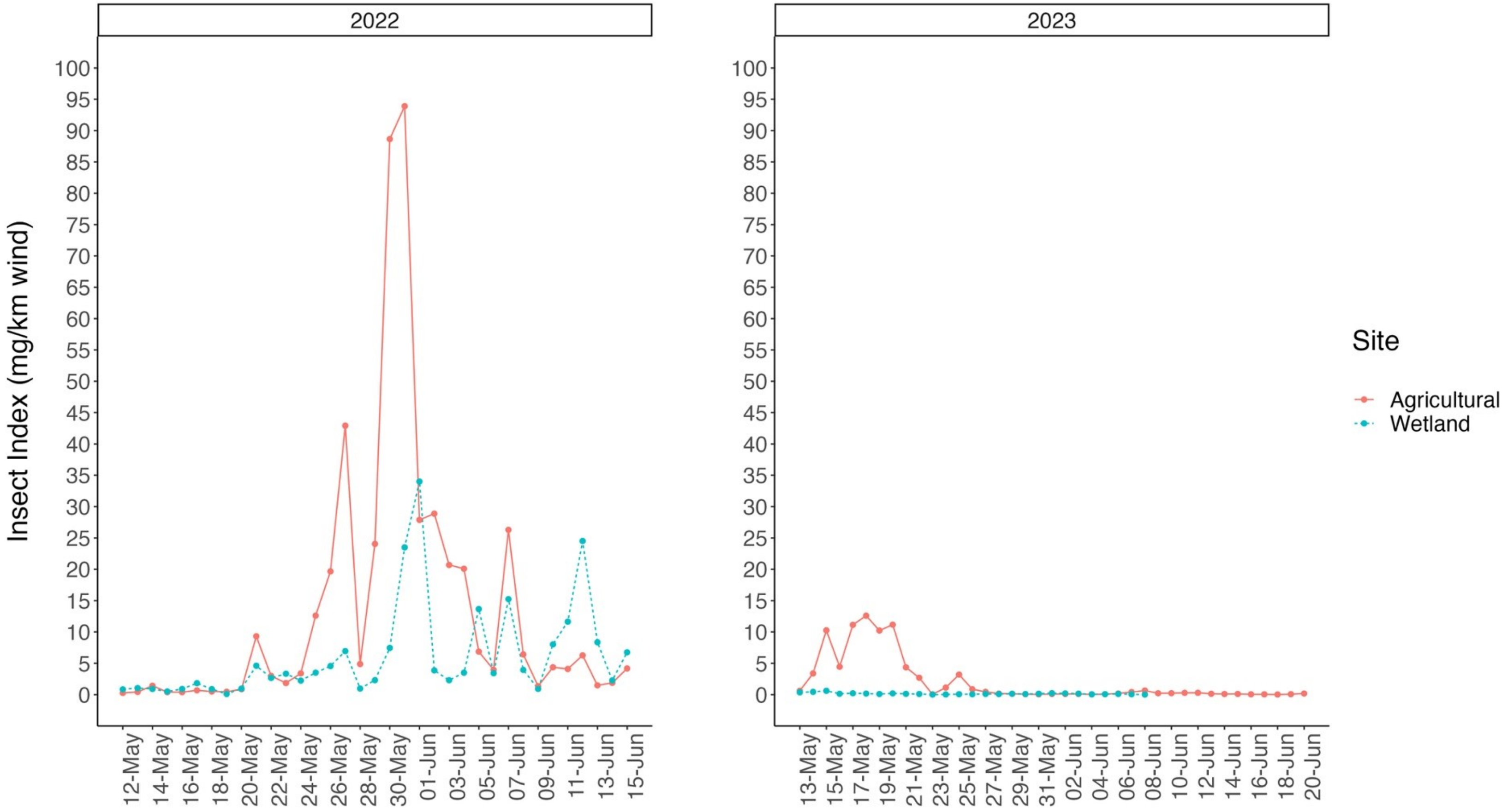


Figure A.1. Daily insect biomass at two study sites (agricultural and wetland) of breeding tree swallows (*Tachycineta bicolor*) near Prince George, British Columbia, Canada, in 2022 and 2023. Insect biomass was measured during the egg formation period and is represented as an index of dry weight (mg) corrected for mean daily wind speed (km/hr).

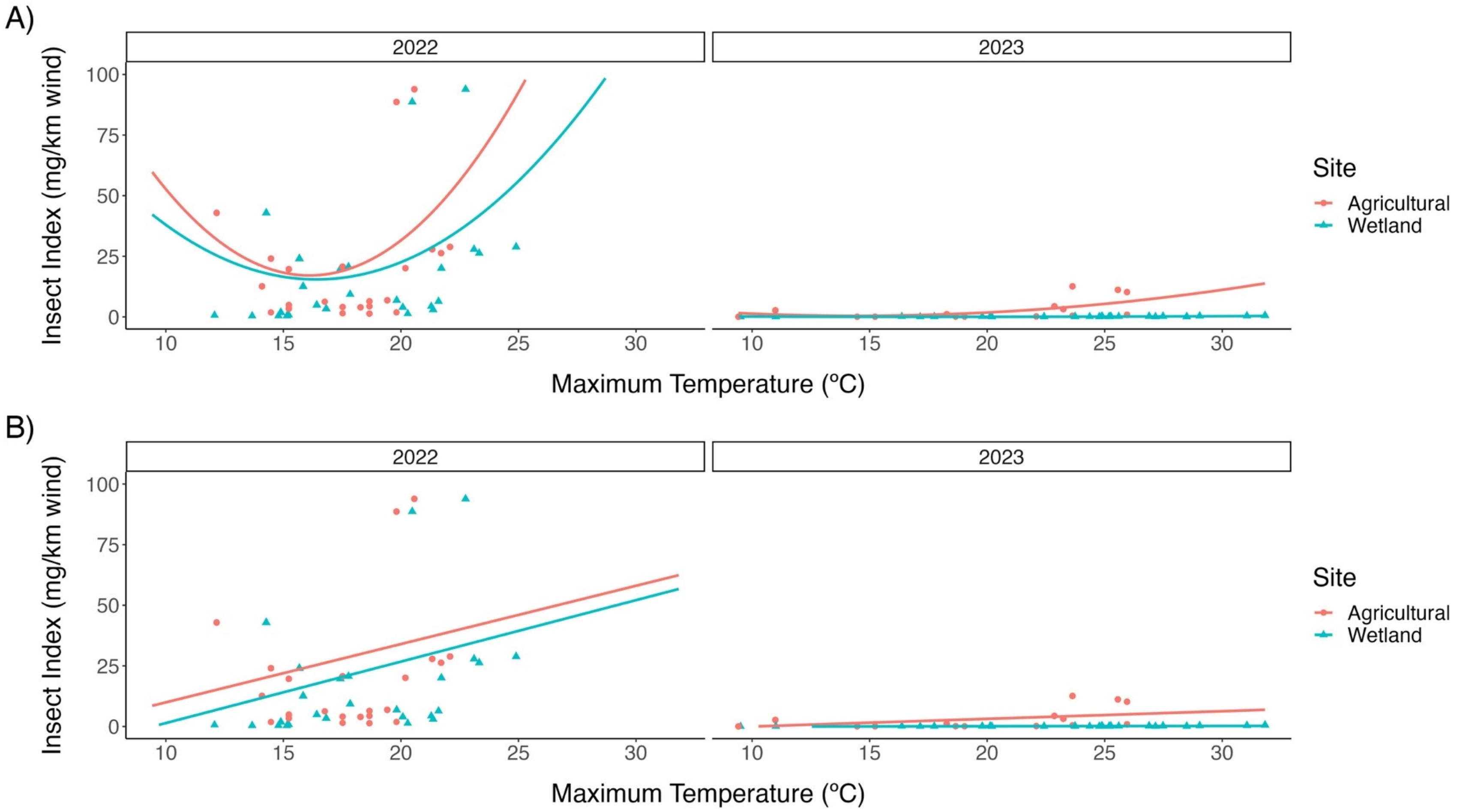


Figure A.2. The quadratic (A) and linear (B) relationships between daily insect abundance and daily maximum temperature (ºC) at two study sites (agricultural and wetland) of breeding tree swallows (*Tachycineta bicolor*) near Prince George, British Columbia, Canada, in 2022 and 2023. Insect abundance is represented as an index of dry weight (mg) corrected for mean daily wind speed (km/hr). The quadratic relationship was significant in 2022 ($\beta = 0.67 \pm 0.17$, $t = 3.94$, $P < 0.001$), and the linear relationship was significant in both 2022 ($\beta = 0.65 \pm 0.11$, $t = 5.99$, $P < 0.001$) and 2023 ($\beta = 0.04 \pm 0.01$, $t = 6.14$, $P < 0.001$; Figure 2B).

A.4 DISCUSSION

Passive aerial tow nets have been used to sample aerial insects since the 1950s (Johnson, 1950), and in research on tree swallows since the 1980s (Quinney & Ankney, 1985; Quinney et al., 1986). The results of my aerial insect sampling, however, were inconsistent with results from other studies and biologically implausible for reasons described hereafter. The biomass of insects sampled at my study sites was significantly greater in 2022 than 2023 (Figure A.1) and at the agricultural site than the wetland site (Table A.1). Interannual variation of aerial insect biomass is common (e.g., Dunn et al., 2011), but the discrepancy in biomass between habitat type is surprising. Tree swallows commonly breed in close proximity to wastewater treatment ponds and other managed wetlands, due in part to the abundance of aerial insects with aquatic larval stages at these sites (Dods et al., 2005; Fernie & Letcher, 2018; Godwin et al., 2019). They also selectively breed in wetland habitat in comparison to intensively managed agricultural habitat (Harris et al., 2024), which may indicate the relative importance of foraging closer to wetlands. Quinney & Ankney (1985) sampled insects in passive aerial tow nets at two sites analagous to my study sites, a municipal sewage lagoon site and a field site (composed of cultivated and uncultivated fields), and found that insect biomass was significantly higher at the sewage lagoon site. I expected to demonstrate similar results at my study sites, especially because tree swallows consistenly breed earlier at the wetland site than the agricultural site, presumably due in part to superior prey quality and quantity (Chapter 3; Dawson, unpublished data). The results presented here therefore seem biologically inexplicable, and raise a question as to whether insects at the wetland site were appropriately captured relative to their actual abundance in the air column. Additionally, the annual emergence of *Bibionidae* at the agricultural site may skew the comparison between the two study sites if this family of flies is substantially easier to capture in tow nets than the taxa present at the wetland site.

Despite a strong relationship between the daily maximum temperature two days before egg laying and the mass of eggs laid in my study (Chapter 3), egg mass was not predicted by the insect biomass on site two days prior to egg laying. Tree swallows are “income breeders”, rapidly using nutrients to form eggs shortly after consuming food (Jönsson, 1997), and insect abundance during the egg formation period has been positively correlated with clutch size, egg mass, and duration of incubation in previous studies (Hussell & Quinney, 2008; Nooker et al., 2005). When the linear relationship between insect biomass and maximum temperature was tested, these two variables were positively correlated in both study years. When the quadratic relationship was tested, insect biomass and maximum temperature were significantly related in 2022; however, the parabola opened upward, indicating that insect availability was higher at more extreme temperatures. Biologically, aerial insect abundance is lower at colder ambient temperatures (e.g., Winkler et al., 2013) and may also be lower at hotter ambient temperatures (e.g., Garrett et al., 2022).

These inconsistencies may be due to an inability of the tow net samplers to collect insect samples that accurately reflect insect availability in the surrounding air column, as has been documented in previous research on tree swallows. Quinney & Ankney (1985) sampled boluses that adults were delivering to nestlings, and demonstrated that the tow nets at their field site caught fewer non-nematoceran dipterans (e.g., Brachycera) and more homopterans than were present in the diet samples. Furthermore, the tow nets captured significantly fewer larger prey items than were being fed to nestlings (Quinney & Ankney, 1985). Similarly, McCarty & Winkler (1999) compared the composition of boluses to aerial insects caught in passive suction samplers (design described by Macaulay et al., 1988) and found that adult tree swallows captured more Odonata and brachyceran Diptera, and generally larger insects, than the samples in the traps. These differences between the composition of boluses and of trapped insects may occur for one or a combination of two reasons: 1) adult tree swallows are selectively foraging for larger prey and of specific taxa to feed to their nestlings than is available in the surrounding air column, and 2) the passive samplers used in these studies do not capture a proportional representation of insects available, and are biased toward capturing insects with specific characteristics, such as body morphology, flying behaviour, or size. Additionally, whereas tree swallows frequently forage within a height of 2 m above ground (i.e., the height of the tow nets), they also spend a considerable amount of time foraging up to 12 m, and occasionally higher (McCarty & Winkler, 1999), so the tow nets may not have collected insects available from all heights at which tree swallows consistently forage.

Researchers studying tree swallows have collected data on the type and size of insects adults typically feed their nestlings, but information is lacking about the prey used by breeding females during the egg formation period. This is an important time period, as aerial insects are scarcer earlier in the breeding season (Dunn et al., 2011) and females are forgaging for both themselves and future embryonic development of their eggs (Williams, 2012). Breeding females may therefore forage for different insect taxa and prey sizes than they would feed their nestlings, and the composition of their diet during the egg formation period may not be well-represented by passive aerial samplers or data collected on prey delivered to nestlings. The species and abundances of insects available and captured by tree swallows may also be strongly site-specific (Dunn et al., 2023). For example, Harris (2023) found that the family *Bibionidae* was not in the top 15 abundant families detected in DNA sequencing of fecal samples from both adult and juvenile tree swallows breeding in the Prairie Pothole Region of Saskatchewan, Canada. Conversely, *Bibionidae* were abundant at my agricultural field site and presumably a likely important component of the diet of tree swallows breeding at this site, although I am unable to confirm if the proportion of *Bibionidae* consumed by breeding females is represented by the proportion caught in the tow nets.

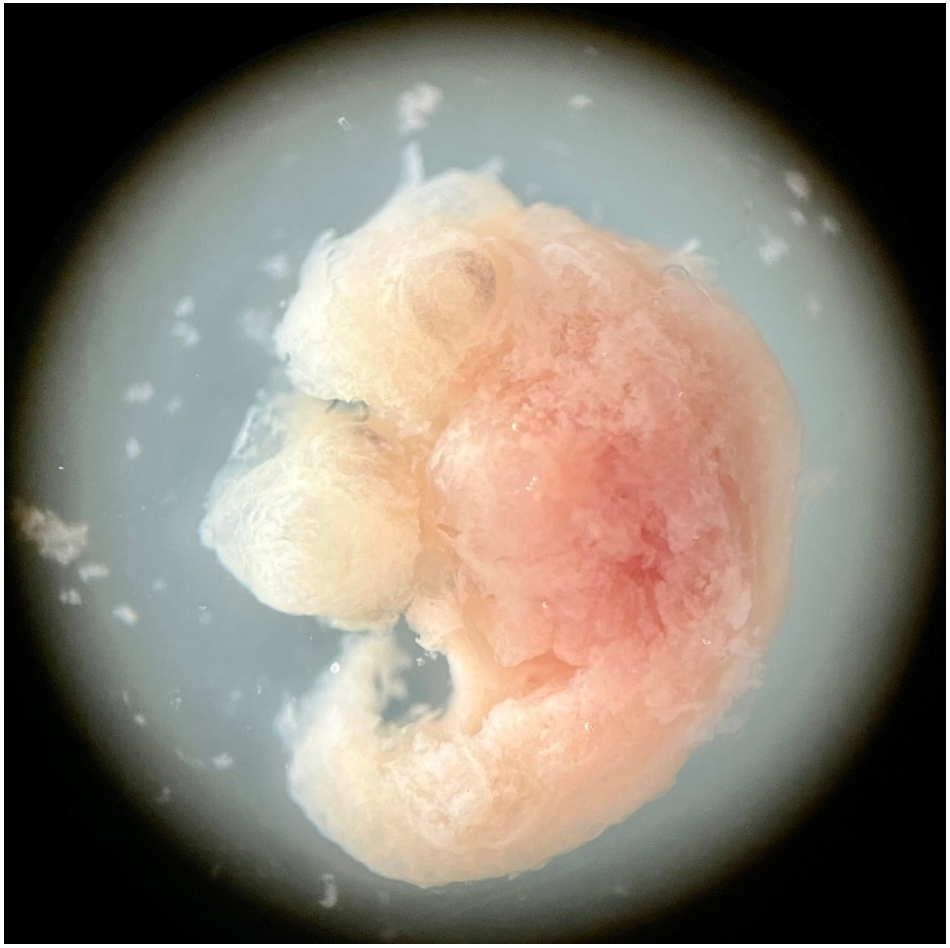
Despite the limitations of this sampling method, passive aerial tow nets have been successfully used by tree swallow researchers to demonstrate the positive relationship between insect biomass and the quality of nestlings; however, the mean insect biomass for 10 or 14 days of the nestling period was used instead of a daily insect index (e.g., Berzins et al., 2021; Harriman et al., 2014; Harriman et al., 2017). Insect abundance in these studies was therefore calculated as a broad moving window that would be less likely to be affected by day-to-day variation. Dunn et al. (2011) similarly used insect biomass data pooled from five days prior to egg laying to assess the relationship between egg laying dates and insect availabiliy and found that egg laying was initiated earlier when mean insect biomass during the egg formation period was greater. It is important to consider, however, that while tree swallows form eggs five to six days prior to laying (Ardia et al., 2006c), rapid yolk deposition (RYD) is not equal throughout the egg formation period. The majority of yolk is deposited in the second half of RYD (Ojanen, 1983), and insect availability two to three days prior to egg laying predicts the mass of eggs laid by tree swallows (Ardia et al., 2006c). It is therefore important to use a fine window of insect data when evaluating the effects of prey abundance during the egg formation period, and notably, Ardia et al. (2006c) conducted their aforementioned fine-scale research using a suction sampler rather than a tow net. Because the results of the relationship between maximum temperature and egg mass in my study align with the findings of Ardia et al. (2006c), I expected insect biomass two days prior to egg laying to be positively related to egg mass. Due to the lack of relationship between these two variables and further inconsistencies in the results of my aerial insect sampling, I did not use insect biomass as a predictor variable for embryonic viability in Chapter 3. While more information is required to understand the provisioning of eggs by breeding female insectivores, researchers should approach the use of passive insect sampling cautiously to ensure the data collected by samplers are an accurate representation of insects available in the air column as well as the diet of the study species.

Appendix B: Summary of predictor variables measured in Chapter 3

Table B.1. Summary of predictor variables used in generalized linear mixed effects models evaluating prenatal reproductive success of female tree swallows (*Tachycineta bicolor*) breeding in 2022 and 2023 near Prince George, British Columbia, Canada. Continuous variables are reported as range; mean ± 1 standard deviation. A total of 653 were included in this study.

Predictor variable	Type (units)	Summary	Description
Study site	Categorical	Agricultural: 298 eggs Wetland: 355 eggs	Study site at which the egg was laid (agricultural or wetland).
Year	Categorical	2022: 405 eggs 2023: 248 eggs	Year the egg was laid (2022 or 2023).
Relative clutch initiation date	Continuous (days)	1 – 26; 10.21 ± 0.21	Difference between when the first egg of the clutch was laid and one day prior to the first egg laid of the year at either study site in each year.
Minimum female age	Continuous (years old)	1 – 4; 2.27 ± 0.04	Minimum age of the female who laid each egg, determined by plumage colour and banding information.
Egg mass	Continuous (g)	1.00 – 2.22; 1.74 ± 0.01	Mass of the egg one day after it was laid.
Lay order	Continuous (clutch position)	1 – 7; 3.61 ± 0.07	Sequential position in which the egg was laid within the clutch. Four eggs laid in eighth position were conservatively assigned a lay order of 7.
Max temp T-2	Continuous (ºC)	9.42 – 31.82; 19.56 ± 4.87	Maximum daily temperature at the study site two days before the egg was laid.
Feather colour	Continuous (unitless)		The first two principal components of a principal component analysis evaluating the spectral reflectance of rump feathers from each female. Higher scores of PC1 are indicative of bluer feathers and higher scores of PC2 are indicative of brighter feathers.

Appendix C: Image of conjoined twin tree swallow embryos



Appendix C.1. Conjoined twin tree swallow (*Tachycineta bicolor*) embryos dissected from an unhatched egg laid in 2022 at a wetland site near Prince George, BC, Canada. The twins share a torso and tail and have separate heads. They died during middle development, five to six days after incubation was initiated. This is the first case of twins to be reported in tree swallows and was the only case identified during the 2022 and 2023 breeding seasons.

Appendix D: Summary of saturated model sets in Chapter 4

Table D.1. Summary of response and predictor variables and associated datasets used in generalized linear effects and generalized linear mixed effects models evaluating laying skips and prenatal reproductive success following a cold snap in 2023 of female tree swallows (*Tachycineta bicolor*) breeding near Prince George, British Columbia, Canada. Models 1, 2, 5, and 6 were fit with binomial regressions and models 3 and 4 were fit with linear regressions. Models 4 – 6 included female identity as a random effect. Clutch initiation date was square-root transformed.

Response variable	**Predictor variables**	**Dataset**
1. Occurrence of at least one laying skip (yes/no) – all ages	Clutch initiation date, clutch size, minimum female age (years), wing chord (mm), head-bill length (mm), body condition[1], study site	Females of all ages who were undergoing the critical period of rapid yolk development (RYD) of at least one egg during the cold snap (n = 52)
2. Occurrence of at least one laying skip (yes/no) – ASY females	Clutch initiation date, clutch size, minimum female age (years), wing chord (mm), head-bill length (mm), body condition[1], study site feather reflectance PC1 and PC2[2]	ASY females who were undergoing the critical period of RYD of at least one egg during the cold snap (n = 40)
3. Number of laying skips (days) – all ages	Clutch initiation date, clutch size, minimum female age (years), wing chord (mm), head-bill length (mm), body condition[1], study site	Females of all ages who were undergoing the critical period of RYD of at least one egg during the cold snap and had at least one laying skip (n = 23)
4. Post-cold snap egg mass	Clutch initiation date, minimum female age (years), max temp T-2[3] (ºC), laying order, female with a laying skip (yes/no), study site	Eggs laid following a cold snap by females who were undergoing the critical period of RYD (two days prior to egg laying) for at least one egg during the cold snap (n = 181)
5. Embryonic viability (hatched/failed to hatch)	Clutch initiation date, minimum female age (years), egg mass (g), laying date, lay-to-set interval (days), study site	Embryonic viability of all eggs that may have been affected by the cold snap (i.e., were being incubated, awaiting the onset of incubation, or were undergoing RYD during the cold snap; n = 192)
6. Embryonic viability (hatched/failed to hatch)	Clutch initiation date, minimum female age (years), egg mass (g), laying date, lay-to-set interval (days), study site, female with a laying skip (yes/no)	Embryonic viability of eggs laid by females that may have responded to the cold snap with a laying skip (i.e., at least one egg in each clutch was undergoing RYD during the cold snap; n = 186)

[1] Calculated by deriving the residuals from a regression between body mass and length of the head-bill, with the number of days following hatch that the measurements were obtained as a covariate from all females included in the study; [2] Higher scores of PC1 are indicative of bluer feathers and higher scores of PC2 are indicative of brighter feathers. [3] Maximum daily temperature two days prior to egg laying.