How do changes in ambient temperature affect House Sparrow (*Passer domesticus*) egg size, coloration, and developmental success?

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Biographical Sketch

My name is Julianna Carpenetti, and I am a Biology (A.B.) major and Psychology minor at Lafayette College. I began conducting research the summer after my first year with Dr. Mike Butler through the Science Horizons program. We investigated how varying degrees of immune challenge affected tree swallow nestling physiology and house sparrow parental feeding. We also collaborated with Dr. Sarah Knutie from University of Connecticut and helped with her nest parasitism research by collecting nests for her continent-wide study. I continued to conduct independent research with Dr. Butler during my sophomore year and I presented a poster at the Society for Integrative and Comparative Biology Annual meeting in Tampa, Florida in January 2019. During the summer of 2019, I continued my field research with Dr. Butler as an Excel Scholar. My favorite aspect of working with animals was seeing how the environment affected them in real time, particularly during periods of warmer or cooler temperatures than usual. This fascination inspired my thesis investigating how these fluctuations in temperature affected egg and nestling development.

Aside from research, I am a PARDner, Orientation Leader, and, as of this year, an EcoRep in the Office of Sustainability. I did not originally expect to pursue research during my undergraduate years, but my experiences have provided me with critical thinking skills, problem-solving techniques, and a hunger for knowledge that I would not have otherwise obtained. I am very interested in behavior in both animals and humans, and my biology and psychology education has helped me to explore those avenues further. Next year, I will be combining my passion for helping others with my interests in behavior by pursuing an M.Ed. in Counselor Education at Penn State University.

Abstract

Egg-laying as a form of reproduction has evolved to display great phenotypic variation based on the organism's environment. In response to environmental challenges, animals have been selected to produce eggs with a wide range of sizes, shapes, and colors, and each of these traits impact the egg and subsequent nestling's developmental success. During the breeding season, the mother will invest resources into her eggs, but the quality and quantity of these resources will depend on what is available in her immediate environment. Ambient temperatures can greatly affect the local food supply, which will impact what the mother can invest in both herself and her offspring. My thesis project examined how these challenges affect house sparrow eggs and nestlings and what temperature metrics are the most impactful on breeding and development. I used a four-year dataset that included breeding data from 290 nests and 3,912 photos of 1,304 eggs, and then analyzed the eggs' size and coloration. I compared these variables to multiple temperature metrics during the pre-laying, incubation, and nestling periods. The results revealed that warmer temperatures correlated with larger eggs, brighter eggshells, and red-shifted eggshell hue values. The eggs were more likely to hatch when temperatures were warmer during the incubation period, and the nestlings were more likely to fledge when the temperature was warmer than historical temperatures for a given nestling period. These results suggest that warmer temperatures elicit greater environmental resource availability, and that house sparrows can readily adapt to fluctuations in temperature in their environment. Further, house sparrow success in warmer temperatures suggests that, as climate change progresses, they will continue to perform well as a species and potentially outcompete less-adaptable species in the same area.

Introduction

Laying eggs is a common reproductive method found in insects, fishes, reptiles, and birds. The evolution of egg-laying as a form of reproduction was an adaptation that allowed multicellular organisms to shift from the aquatic realm and into terrestrial life (Wolpert and Szathmáry, 2002). With this transition, species had to respond to the lack of water which elicited varied methods of reproducing, respiring, and eating (Ashley-Ross et al., 2013), leading to diversification in egg anatomy and, hence, its functionality (Kilner, 2006). The egg structure provides several practical advantages such as increased protection, thermoregulation, internal environment control, and camouflage (Lahti and Ardia, 2016; Sun et al., 2012). Phenotypically, eggs can vary in terms of their size, shape, mass, and coloration (Patten, 2007**.** These variations can fluctuate based on resource availability at the time of development, which directly influences the quality of the egg and effectiveness against stressors such as predation (Patten, 2007) and inclement weather (de Zwaan et al., 2020). Over evolutionary time, the appearance and relative egg strength respond in ways that accommodate the local environmental demands, such as the level of camouflage required by the nest's location as well as eggshell thickness to combat the sun's rays and temperature changes (Kilner, 2006).

Many aspects about the quality of the egg and subsequent nestling can be inferred by the egg's appearance. Eggs with a greater shell surface area may be able to better regulate water and gas permeability, especially in response to environmental changes (Portugal et al., 2010). This can be attributed to larger pores in the egg or more of them, both of which may be associated with eggs with greater mass and eggshell thickness (Zimmermann and Hipfner, 2007). Larger eggs also tend to lead to nestlings with a greater mass, which are more likely to survive into the breeding season (Amat et al., 2001; Styrsky et al., 1999). However, larger eggs also require greater parental energetic investment both before and after laying (Williams, 2005). If the size of the egg requires too much energy or too many resources from the mother, the likelihood of survival of both the embryo and the mother could decrease (Kölliker, 2012).

While an egg's size can have an influence on the future embryo's development, eggshell color and patterning can also have an impact. In avian species, in particular, the eggshells are calcified (Patten, 2007). Calcium allows for a stronger shell that is thicker and less porous, better protecting the embryo inside (Graveland and Drent, 1997). The presence of calcium in eggshells also acts as a matrix allowing for more pigmentation on the surface (Gosler et al., 2000; Patten, 2007). The two pigments responsible for much of the coloration in eggs are protoporphyrin and biliverdin, creating brown and blue-green hues, respectively (Brulez et al., 2014; Gosler et al., 2011). Beyond functions such as camouflage and sexual signaling of maternal fitness (López de Hierro and De Neve, 2010), protoporphyrin can aid in minimizing internal water loss and reducing solar damage (Gosler et al., 2005). Clusters of protoporphyrin are linked to stronger eggshells, likely due to the calcium present in those areas (Graveland and Drent, 1997; Hargitai et al., 2016). Protoporphyrin and biliverdin also provide strength and flexibility to the eggshell, which is very important when considering the unpredictability of weather when living in an outdoor environment (Kilner, 2006). However, eggshell coloration can also result in costs to the developing embryo, as eggs with darker pigmentation are more at-risk of overheating in the sunlight as they cannot independently thermoregulate (Kilner, 2006). Parents are responsible for maintaining the internal temperature within the eggs during with incubation period, but, if the ambient temperatures are too high or there is excessive solar radiation, the eggs can overheat (Gómez et al., 2016).

The source of eggshell pigmentation in the mother is not entirely clear, but is thought to be deposited into the eggshell while developing in the shell gland (Hargitai et al., 2017). Further, there is strong support in the canary (*Serinus canaria*) (Hargitai et al., 2016), blue tit (*Cyanistes caeruleus*) (Puente et al., 2007), and pied flycatcher (*Ficedula* hypoleuca) (Moreno et al., 2005) that the mother's condition is largely responsible for the variation in pigment that can be found in the eggshells. Lower maternal quality has been linked to darker spots of protoporphyrin on the eggshells, suggesting that the mother has a nutrient-poor diet and lower antioxidant capacity (Hargitai et al., 2016). However, evidence is mixed regarding protoporphyrin's links to female quality, as spots of protoporphyrin can get darker or remain the same in successive clutches, suggesting that the mother can readily produce protoporphyrin in sufficient supply (López de Hierro and De Neve, 2010).

The protoporphyrin and biliverdin responsible for providing coloration in eggshells must come from the mother during the egg's development, which is a resource that must be distributed amongst her whole clutch. In order to provide these resources to the eggshell and offspring, a mother can allocate her own resource supply in a variety of ways, including pulling from her own nutrient stores, redirecting her energy away from her own metabolism and towards offspring development, or by substantially increasing her dietary intake (Houston et al., 1995). The most viable of the three options would be influenced by the mother's current condition as well as her local environment and its resource availability. The tendency to ultimately use her own stored supply or to pull from what is readily available in the environment also depends on if the mother is a capital breeder or income breeder. Capital breeders accumulate and save resources prior to reproduction and allocate those resources to the breeding attempt. Income breeders, however, readily ingest and allocate resources to put immediately toward reproduction (Sainmont et al., 2014). In the former, the current environmental food and resource availability has minimal effect on the quality of the offspring the mother can produce, whereas the latter is heavily impacted by the current local resource supply (Sainmont et al., 2014). Therefore, the environmental conditions leading up to and during the laying period are critical for income breeders, as the availability of nutritious and calcium-rich foods can heavily impact the success of offspring (Graveland and Drent, 1997).

While a mother's stores of calcium and other nutrients are limited, additional stress is put on her body as the investment into her eggs increases (Russell et al., 2008). Maternal energetic investment towards her offspring could compromise her ability to properly care for these eggs if the effort shifts nutrients and energy away from what she individually requires (Gullett et al., 2015; Russell et al., 2008). While the overall success of the egg partially depends on the eggshell quality, it is also affected by the mother's investment post-laying during the incubation and nestling periods. During these critical periods of development, the eggs and nestlings are still very dependent on their parents for food, protection, and warmth (Lowther and Cink, 2020). The incubation period begins once all eggs have been laid (Lowther and Cink, 2020). The incubation period then lasts until all of the eggs have hatched and its duration can be influenced by the level of parental investment and the quality of eggshell characteristics (Lowther and Cink, 2020; Zimmermann and Hipfner, 2007). The nestling period begins when all of the eggs have hatched and the offspring begin to develop under the care and protection of their parents (Lowther and Cink, 2020). During this time, the nestlings begin to grow as well as develop more complex functions, such as thermoregulation, that will allow for them to successfully fledge, or leave the nest (Węgrzyn, 2013).

Another stressor that can impact maternal investment into her offspring is the environment, including changes in weather patterns (Fletcher et al., 2013; Gullett et al., 2015). The weather, in terms of temperature, both before and during the breeding season is largely responsible for the productivity of that season, with its effects being seen in resource availability and quality (Amat et al., 2001), migration patterns, the time when the egg-laying period is initiated (Glądalski et al., 2014), and population maintenance and growth (Pearce-Higgins et al., 2015). Further, temperature variations can also impact the local food supply. The quality and quantity of food the mother can access will act as the nutrient basis that will be invested into her offspring. If a mother has plentiful resources, she would be able to pass more of those resources onto her offspring, which would translate to greater egg size and pigmentation in the form of protoporphyrin and biliverdin, all while still keeping some resources for herself (Kölliker, 2012). Conversely, if a mother's available resources are compromised as a result of poor weather conditions and limited resources, her ability to invest energy into her eggs may be reduced (Fletcher et al., 2013; Gullett et al., 2015).

When associating changes in temperature with resource availability, it is important to consider that the recent rise and variation in temperature can be interpreted within the context of climate change (Gullett et al., 2015). The top ten hottest years ever recorded have all occurred since 2005 (Lindsey and Dahlman, 2021), supporting that global warming and climate change are very much in effect. Further, the combined land and ocean temperature has increased consistently each decade since 1880, but the average rate of increase doubled in 1981, with the temperature rising 0.18° Celsius each decade (Lindsey and Dahlman, 2021). While temperatures are likely to continually rise on this trend, there is also an expectation that there will be an increase in extreme weather events (Glądalski et al., 2014). These changes in climate are likely to affect breeding patterns, as egg-laying can be advanced or delayed as a result of temperature changes that are much warmer or cooler than usual, respectively (Glądalski et al., 2014). These changes in temperature can create living conditions that are inadequate for small prey such as insects and invertebrates, as well as decrease the availability of seeds and grass, which all largely constitute a bird's diet (Lowther and Cink, 2020). When a mother's prey is no longer available or very limited, she has to determine how to distribute and consume these resources with her offspring (Kölliker, 2012). This trade-off could be demonstrated by having smaller eggs to lower her reproductive energetic expenditure or laying fewer of them (Kamel and Williams, 2017). These eggs may be less spotted in order to distribute the calcium and nutrients amongst all the eggs as well as keep some of these resources to continually sustain herself (Kamel and Williams, 2017). Under ideal conditions, the mother would be able to produce eggs with uniform size and nutrient content, but environmental constraints can result in an uneven distribution of the mother's resources in her offspring (Kamel and Williams, 2017).

My study subject, the house sparrow (*Passer domesticus*), allows for an examination relating variation in temperature to variation in eggshell appearance. House sparrows can lay up to eight eggs in a single clutch and breed multiple times during their nesting season (Lowther and Cink, 2020), allowing for a robust sample size. Their eggs are typically off-white with a slightly blue hue comprised of biliverdin with scattered brown spots of protoporphyrin, which allows for great variation in appearance and, by extension, varying strength and protection both between and within clutches (López-de-Hierro and Moreno-Rueda, 2010). This speckled pattern most likely evolved in response to the need for the eggs to camouflage within their environment, which is located in cavities in trees, infrastructure, and bird-specific boxes (Kilner, 2006). However, since cavities typically provide considerable protection and cover from the external weather conditions and predators, the persistence of speckled eggshells in house sparrows suggests that pigmentation has an appreciable functional role (Kilner, 2006).

My research was designed to determine the relationship between variation in air temperatures and its effects on house sparrow egg size, egg appearance, hatching success, and subsequent fledging success, framed in the context of global climate change. The study examined these variables during the pre-laying period, the incubation period, and the nestling period of house sparrow development over a four-year period. I hypothesized that when temperatures were more similar to the historical average during the pre-laying period, the eggs would be larger and have more pigmentation in the eggshell (Kamel and Williams, 2017). Further, I predicted that these eggs would be more likely to hatch, and the nestlings would be more likely to fledge (Gullett et al., 2015). I expected that these patterns would be the result of increased availability of seasonally nutritious food, resulting in greater reproductive success (Fletcher et al., 2013). Consequently, I also hypothesized that pre-laying period temperatures that were less similar to historical averages would result in smaller, less pigmented eggs, which were less likely to hatch and to fledge, assuming the mother's access to resources would be compromised (Kamel and Williams, 2017; Kölliker, 2012). Further, I also predicted that when the temperature during the incubation period was closer to the historical average, the eggs would be more likely to hatch. Similarly, I expected that when the temperature during the nestling period were closer to the historical average, the nestlings would be more likely to fledge. In both of these cases, I expected that the parents would not have temperature as an obstacle when caring for and feeding their offspring, leading to more successful outcomes.

Methods

Study site

I completed this study using photographs of house sparrow (*Passer domesticus)* egg and breeding data collected from a field site at Metzgar Fields Athletic Complex in Easton, PA. Metzgar Fields Athletic Complex is comprised of 80-acres of land, containing various athletic fields and practice facilities, small forested areas, and open land. Eighty-three nest boxes with a 1.5-inch diameter hole (Kinsman Company, 1B-38) have been arranged around the approximately 1.5-mile perimeter of the complex. While these nest boxes are typically occupied by house sparrows, tree swallows (*Tachycineta bicolor*), eastern bluebirds (*Sialia sialis*), and house wrens (*Troglodytes aedon*), this study focuses on house sparrow activity. House sparrows build nests and initiate egg-laying from April and continue through August (Lowther and Cink, 2020). During the breeding seasons of 2017 through 2019, members of the Butler lab checked nest boxes daily to document breeding activity. A reduced amount of breeding data were collected in 2020 due to COVID-19 constraints. Data from 2020 were included in analyses only if breeding data were known instead of estimated.

All nest boxes were checked twice weekly during the summer breeding seasons, lasting from April to August. I personally collected data during the breeding seasons of 2018 and 2019, and other student researchers and Dr. Butler collected data for the 2017 and 2020 breeding seasons. The nest checks consisted of recording the amount of nesting material in the boxes, counting the number of eggs and nestlings, as well as monitoring and recording the dates in which hatching and fledging activity occur, which is the developmental stage in which offspring begin to leave the nest. The accumulation of nest material inside of a nest box was indicative of nesting activity, potentially leading to egg-laying. If eggs were found in the nest, that nest was considered active and when the number of eggs in the nest was unchanged for 2 days in a row, the clutch was considered complete. House sparrows lay one egg per day, so I could use this fact to determine the date the clutch began by counting backwards from the day the last egg is laid (Badyaev et al., 2002). The incubation period began the day the last egg was laid and lasted until the last egg hatched (Lowther and Cink, 2020). Between the fourth and eighth days of the incubation period, the eggs would be photographed and their mass was recorded to the nearest 0.01g (Butler et al., 2020). Photos were taken in triplicate in an on-site, light-controlled environment (see *Egg photos*, below). In 2017 through 2020, 140 eggs from 32 nests, 427 eggs from 96 nests, 533 eggs from 117 nests, and 204 eggs from 45 nests, respectively, provided the data for my analyses. Overall, I collected size and appearance data from 3,861 photos. I would start to check the nests daily for hatching starting nine days into this period (Butler et al., 2020; Lowther and Cink, 2020). Ten days after the first egg hatched, I began to check the nests for nestling fledging. During this span of time, the nestling period, the nestlings were growing and developing until they were ready to leave the nest on their own. All work with eggs and animals was conducted under IACUC protocols that were approved on 4/19/2016 and 4/11/2019.

Pre-laying period

The pre-laying period commences the seven days prior to the day the first egg is laid in the clutch (Fig. 1). During this week-long period, the mother will collect and process resources to invest in these eggs, and the quality and quantity of these resources will be dependent on the environment. This process of the mother collecting and using the readily available resources in the immediate environment to invest into her offspring is known as income breeding (Sainmont et al., 2014). Income breeders do not accumulate their energy and nutrient stores like capital breeders, so they are dependent on the food they can find in each given breeding season (Stephens et al., 2014). Fluctuations in ambient temperatures during the week leading up to egg-laying will affect the environmental resource supply, impacting the potential success of the clutch working through the mother's ability to find resources (Fletcher et al., 2013; Stephens et al., 2014).

Incubation period

The incubation period was defined as the nine days after the last egg in the clutch was laid. Dr. Butler's field site's 2017-2019 dataset showed that the average incubation period was 10.4 days and the minimum was nine days. By using the minimum number of days as my designated incubation period instead of the average, all of the data during that period would represent eggs and not a mix of eggs and nestlings from early hatching. After nine days, the eggs would typically begin to hatch.

In order to ensure successful hatching, parents work to maintain consistent internal egg temperatures of during the incubation period (Lowther and Cink, 2020). Ambient temperatures that are much higher or lower than the historical average could result in the parents failing to give their eggs adequate attention (Kölliker, 2012). When experiencing these temperatures that are inconsistent with what they are accustomed to, incubating parents have to allocate their energetic reserves to either their own self-maintenance or the development of their offspring (Kölliker, 2012). Furthermore, abnormally warm temperatures could overheat the internal temperature of the nest box, impacting the chance of survival for the eggs or nestlings inside

(Ropert-Coudert et al., 2004). Since the eggs during the incubation period are dependent on the nest box for protection as they develop, they must contend with the increasing demands to thermoregulate while also potentially facing a decrease in parental attention due to the unbearably high temperatures (Węgrzyn, 2013). These challenges can greatly impact the success of the offspring, as they are solely dependent on their parents for care at this stage of development.

Nestling Period

The nestling period is the amount of time that it takes for nestlings to develop and leave the nest after hatching (Kouba et al., 2015). Dr. Butler's field site's 2017-2019 dataset showed that the average time length of the nestling period was 14.9 days and ranged between 11-20 days. For my purposes, the nestling period was defined as the ten days following the first date of hatching to encompass only days when the nestlings were still in the nest. This critical period of development is when the offspring start to develop their plumage, increase in mass, and regulate their body temperature (Andreasson et al., 2016; Lowther and Cink, 2020). The temperature during this period could impact how these nestlings develop, as they may have to divert more of their energy and resources into survival instead of growth (Węgrzyn, 2013). During this time, the mother is responsible for bringing her growing nestlings food, and the quality and quantity of this food will be directly dependent on the state of the local environment (Pearce-Higgins et al., 2015). Similar to the other developmental stages, extreme ambient temperatures can affect the mother both directly through pressures to invest her energy into her own temperature regulation (Ruuskanen et al., 2021) or indirectly through increased resource scarcity and decreased prey availability when foraging (Pearce-Higgins et al., 2015; Williams, 2018). This situation creates a trade-off in which she is must compromise on her own diet and condition in order to care for her offspring (de Zwaan et al., 2020).

Egg photos

During the incubation period, specifically between five and nine days after the clutch was completed, three photos were taken of each egg, which was rotated approximately one-third of the way between photos. Each clutch was removed from its nest and carefully transported to an on-site field station to take these photos. During this time, the nest box entry would be covered to prevent the parents from returning to and then abandoning a disrupted nest (Lund, 2017).

Photos were taken under standardized lighting conditions with a Nikon D70 camera that was mounted above the egg along with a color standard (ColorChecker Passport, X-Rite, Grand Rapids, MI, USA) (Butler and Waite, 2016). Two lightbulbs (Utilitech 7.5-Watt (40W) A19 Medium Base Daylight (5000K) LED) were used to illuminate the photos and the eggs were often cradled on a piece of cotton for protection and to keep it in place. The mass of each egg was also recorded at this time. Upon completing this procedure, the eggs were returned to their nest and the nest box entry was uncovered. I then analyzed the egg photos using two image-processing programs: ImageJ and Adobe Photoshop.

ImageJ protocol

ImageJ was used to determine the dimensions of each egg, including volume and surface area, using the Egg Tools plugin for egg measurement (Troscianko, 2014). Using the triplicate photos, an average of these measurements was taken to represent each egg, and then further averaged for each clutch to give one average measurement for each nest.

For each photo, the egg shape was outlined using the Multi-point tool. Each egg was outlined with 8-12 points, and then the Egg Measurement feature used these points to create an outline of the egg (Fig. 2). When the egg was adequately outlined, I indicated a scale bar on the image. Using the ruler on the color standard in the photo, I measured one millimeter. ImageJ produced a data table for the photo containing the area of the egg in pixels, maximum length, maximum width, volume, ellipse deviation, surface area, and the left and right R-squared values. The maximum length captured the longest vertical stretch on the egg from the top to the bottom and the maximum width captured the widest horizontal stretch on the egg from the left and right sides (Troscianko, 2014). Volume and surface area of the eggs were measured in millimeters cubed and millimeters squared, respectively, and ImageJ accounted for the curvature of the egg for these calculations (Troscianko, 2014). Ellipse deviation measured how the curvature of the egg compared to a standard ellipse, with smaller values representing a pointier egg and larger values representing a rounder egg (Troscianko, 2014). Egg mass, maximum length, maximum width, volume, and surface area were used for determining overall egg size.

Photoshop protocol

With Adobe Photoshop 2020 (ver. 21.2.2), I analyzed the patterning of the eggs to quantify their hue, saturation, and brightness (Butler and Waite, 2016). Each photo was analyzed individually, and then each metric was averaged to give one value for each egg, and then further averaged with the other eggs in the clutch to give one metric for each nest as a grand mean. Using the Object Selection Tool, I outlined each egg (Fig. 3). I repeated this until the whole egg was adequately outlined and the background was not also picked up in the outline. Using this highlighted area, I then used the Color Picker window along with the Histogram feature to determine the hue, saturation, and brightness values from the egg’s median red, green, and blue color values. Each egg’s data were then transferred to a spreadsheet for later analysis.

To ensure standardized color throughout the data, one photo from each clutch was arbitrarily selected to record the hue, saturation, and brightness values for the white, light gray, tan, and brown squares on the color standard. These four colors were selected because they most closely resembled the colors found on the house sparrow eggs that were studied. The white, light gray, and tan squares were selected using the Magic Wand Tool to outline the whole square. The brown square was selected using the Object Selection Tool because it better captured the area than the Magic Wand Tool for that specific color.

Weather data

Weather data were downloaded from the National Oceanic and Atmospheric Administration (NOAA) database. I accessed the maximum, minimum, and observed temperatures for each day of the years 2017, 2018, 2019, and 2020. I also obtained the 30-year historical average for the daily average temperature, daily maximum temperature, and daily minimum temperature for every day of the year. Historical data were not available for the dates 7/11/17, 4/29/18, 6/24/18, 7/8/18, 8/5/18, 5/25/19, and 6/13/19, most likely due to an issue with the weather station. The data were collected at the nearest weather station, located in Phillipsburg, New Jersey, which is approximately six miles from the Metzgar field site. For each nest, I recorded the average temperature, maximum temperature, and minimum temperature for the pre-laying period, incubation period, and nestling period for the years 2017-2020. These temperatures were then compared to the 30-year historical average temperature data (Jiguet et al., 2006).

Statistical analysis

Some nests and eggs were omitted from the analysis due to incomplete data or individual eggs having attached debris that would skew the Photoshop analysis. The final analysis included 3,861 photos of house sparrow eggs, 1,287 eggs, and 290 nests spanning the breeding seasons of 2017, 2018, 2019, and 2020. I then calculated repeatability for egg size metrics (maximum length, maximum width, volume, ellipse deviation, surface area) and color metrics (hue, saturation, and brightness) for the three photos of each egg to test whether photos were accurately capturing egg appearance (Lessells and Boag, 1987). I also calculated repeatability of all eggs within a nest to test whether individual females laid eggs that were more similar to each other than they were to eggs laid by other females (Butler and Waite, 2016; Lessells and Boag, 1987). Average values for eggs across triplicate photos were used in subsequent analyses examining difference among eggs, and average values within a clutch were used for analyses examining differences among clutches.

Because multiple metrics of egg size are correlated with each other, I generated a single metric that captures egg size using a Principle Components Analysis. Using the variables average egg mass, average egg length, average egg width, average volume, and average surface area, we generated a principal component (hereafter, Egg Size) that had an eigenvalue of 4.10 and accounted for 82% of the variation. All other principal components had eigenvalues less than 0.6 and accounted for less than 12% of the variation. Because the first principal component loaded positively and relatively uniformly for all size-related metrics (all eigenvector loadings between 0.40 and 0.49), we used Egg Size in all subsequent analyses to characterize differences in size among eggs.

To test whether egg appearance was related to egg coloration, we ran mixed models with either egg size or egg shape (i.e., ellipticity) as dependent variables, egg hue or egg brightness as independent variables, and nest ID (i.e., nest box within a year) as a random effect. These relationships are not interpreted as causal, but rather correlational.

To test if temperature during the laying period predicted egg size, we ran separate models with egg size as a dependent variable, both a temperature metric and calendar year as fixed effects, and nest ID as a random effect. Each model had a different temperature metric (Table 1) that was applied to the seven-day pre-laying period. We took each model's AIC value, identified the model with the lowest AIC, and examined all models that had AIC values within 2 of the lowest one. We repeated this process twice more, with one suite of models that had average eggshell brightness of the clutch as a dependent variable, and average eggshell hue of the clutch as a dependent variable. Thus, for each of these three dependent variables, we identified the most informative models.

We then used a similar approach to test how multiple metrics, including temperature during the incubation period, predicted hatching success. Because hatching success was non-normally distributed, we categorized hatching success as an ordinal variable, with nests that had hatching success of 0%, 1-99%, and 100% as categories, and then performed generalized linear mixed models with a multinomial dependent variable. The first suite of models had average eggshell brightness of the clutch, average egg size of the clutch, calendar year, the number of eggs laid, and a temperature metric as fixed effects, and nest ID as a random effect. The temperature metrics were the same as those used for the pre-laying period, except this time applied to the nine-day incubation period (Table 1). Similar to above, only the model(s) with the lowest AIC values were interpreted. We also ran a second suite of models with the same set of metrics as above, but rather than using average eggshell brightness for the clutch, we used average eggshell hue.

Lastly, we again performed a similar approach to test how multiple metrics, including temperature during the nestling period, predicted fledging success. Similar to hatching data, fledging data were not normally distributed and we categorized fledging success as an ordinal variable, with nests that had fledging success of 0%, 1-99%, and 100% as categories, and then performed generalized linear mixed models with a multinomial dependent variable. The first suite of these models had average eggshell brightness of the clutch, average egg size of the clutch, calendar year, the number of nestlings that had hatched, and a temperature metric as fixed effects, and nest ID as a random effect. The same temperature metrics used for the pre-laying period and incubation period were also applied to the ten-day nestling period (Table 1). Similar to above, only the model(s) with the lowest AIC values were interpreted. We also ran a second suite of models with the same set of metrics as above, but rather than using average eggshell brightness for the clutch, we used average eggshell hue.

Results

Repeatability, egg size, and coloration

Egg size and coloration metrics were significantly repeatable within each egg (Table 2). For each egg, average values were calculated for each metric and used in subsequent analyses. Within a clutch, egg size and coloration metrics were also significantly repeatable (Table 3). Volume was the most repeatable size metric and hue was the most repeatable coloration metric.

Further, eggshell brightness was positively correlated with egg size ($F_{1,964} = 6.80$, $P = 0.0093$), although eggshell hue was not significantly related to egg size ($F_{1,964} = 0.40$, $P =$

0.5255). Further, ellipse deviation was significantly positively correlated with eggshell brightness ($F_{1,964} = 9.83$, $P = 0.0018$), but not to eggshell hue ($F_{1,964} = 0.40$, $P = 0.5253$) or egg size ($F_{1,996} = 0.07$, $P = 0.7873$).

Pre-laying period temperature on egg appearance

The model that best predicted average egg size in a clutch (Table 4) included the $T_{\text{HistAvgMax}}$ for a clutch's pre-laying period as an independent variable, with historically warmer high temperatures associated with larger eggs ($F_{1,110} = 34.66$, $P < 0.0001$; Fig. 4a). Eggs also were significantly larger when the T_{HistAvg} during the pre-laying period was warmer ($F_{1,110} = 33.44$, $P < 0.0001$; Fig. 4b).

When average temperatures were higher than the historical average (T_{DiffAvg}) during the pre-laying period, females laid eggs with brighter eggshells ($F_{1,108} = 18.39$, $P < 0.0001$; Fig. 5a). Eggshell brightness significantly decreased when both the $T_{\text{HistAvgMax}}$ ($F_{1,108} = 17.96$, $P < 0.0001$; Fig. 5b) and the T_{HistAvg} during the pre-laying period were warmer ($F_{1,108} = 16.66$, $P < 0.0001$; Fig. 5c).

Average eggshell hue in a clutch was best predicted by a significant negative relationship with T_{Max} during the pre-laying period, such that eggshell hue was red-shifted when the temperature was warmer ($F_{1,108} = 18.23$, $P < 0.0001$; Fig. 6a). Eggshell hue was significantly red-shifted when both the $T_{\text{HistAvgMax}}$ ($F_{1,108} = 18.64$, $P < 0.0001$; Fig. 6b) and the T_{HistAvg} of the pre-laying period were warmer ($F_{1,108} = 17.46$, $P < 0.0001$; Fig. 6c).

Incubation period temperature on egg appearance and hatching success

All models included average egg size and number of eggs as independent variables, plus an additional metric related to eggshell coloration. When the eggshell coloration metric was brightness, a larger proportion of eggs hatched in clutches that were incubated when $T_{\mathrm{HistAvgMin}}$ was warmer ($F_{1,104} = 4.30$, $P = 0.0406$; Table 5; Fig. 7a). Clutches had significantly lower hatching success when the $T_{\mathrm{DiffAvgMin}}$ for the incubation period was greater ($F_{1,104} = 4.00$, $P = 0.0481$; Fig. 7b). Further, a larger proportion of eggs hatched when the T_{AvgMax} ($F_{1,104} = 4.19$, $P = 0.0432$; Fig. 7c), the T_{HistAvg} ($F_{1,104} = 4.12$, $P = 0.0448$; Fig. 7d), and the $T_{\mathrm{HistAvgMax}}$ ($F_{1,104} = 3.93$, $P = 0.0500$; Fig. 7e) were warmer during the incubation period. There was no significant relationship found between hatching success and the T_{Avg} ($F_{1,104} = 3.12$, $P = 0.0802$; Fig. 7f).

When the eggshell coloration metric was hue, a larger proportion of eggs hatched in clutches that were incubated when $T_{\mathrm{HistAvgMin}}$ was warmer during the incubation period ($F_{1,104} = 5.39$, $P = 0.0223$; Table 5; Fig. 7a). Hatching success significantly increased when the T_{AvgMax} ($F_{1,104} = 5.14$, $P = 0.0254$; Fig. 7c), the T_{HistAvg} ($F_{1,104} = 5.22$, $P = 0.0244$; Fig. 7d), and the $T_{\mathrm{HistAvgMax}}$ during the incubation period was higher ($F_{1,104} = 5.03$, $P = 0.0270$; Fig. 7e). There was also an increase in hatching success when the T_{Avg} during the incubation period was higher, but this relationship was not significant ($F_{1,104} = 3.90$, $P = 0.0509$; Fig. 7f). Additionally, as the $T_{\mathrm{DiffAvgMin}}$ increased, hatching success significantly decreased ($F_{1,104} = 5.00$, $P = 0.0275$; Fig. 7b).

Nestling period temperature on egg appearance and fledging success

Fledging success was best predicted by its significant positive relationship with the $T_{\mathrm{DiffAvgMax}}$ for the nestling period ($F_{1,84} = 28.72$, $P < 0.0001$; Table 6; Fig. 8a), when controlling for average eggshell brightness and average egg size. When controlling for average eggshell hue and average egg size, fledging success was also best predicted by its significant positive relationship with the $T_{\mathrm{DiffAvgMin}}$ ($F_{1,84} = 28.53$, $P < 0.0001$; Fig. 8b).

Discussion

Egg appearance

Overall, warmer temperatures during the pre-laying period were associated with larger eggs. This result did not support my hypothesis that warmer temperatures would bring about smaller eggs. I predicted that warmer temperatures would negatively affect the local food supply, which would cause the mother to consume a lower quality or quantity of food. However, an opposite mechanism may have been transpiring during periods of warmer temperatures, in which the food supply could thrive and become more abundant and nutritious (Vatka et al., 2011). Alternatively, egg size may be related to temperature through a mechanism other than food supply. In this case, egg size would have been affected by another factor, such as the mother's condition and physiology (D'Arpa et al., 2021; Ebneter et al., 2016).

Temperature also affected eggshell formation in terms of pigmentation. Eggshell brightness was best predicted when the pre-laying period temperature was greater than the mean historical temperature. In other words, eggshells were brighter (i.e., had lower levels of pigmentation) when the pre-laying period temperatures were warmer than normal. The pigmentation in eggshells comes from primarily protoporphyrin as well as some biliverdin, which are both found in house sparrow eggs (López de Hierro and De Neve, 2010). Since protoporphyrin elicits a red-brown color, commonly recognized as the spots found on house sparrow eggs, and pigments absorb light, more protoporphyrin on an eggshell would result in lower brightness values (Walters and Getty, 2010). Brighter eggs would be assumed to have less protoporphyrin, as red-brown colors would decrease brightness by absorbing more light.

However, eggshell hue was red-shifted as temperatures during the pre-laying period increased. Because red-shifted hue values would be associated with more brown, protoporphyrin-based spotting on the egg's surface, these results initially appear contradictory with the decreased pigmentation in (i.e., brighter) eggshells laid during warmer periods. However, brightness captures information related to the total amount of pigmentation, whereas hue captures the relative amount of different types of pigments. Thus, a red-shifted hue means that there was relatively more red-brown protoporphyrin than biliverdin, regardless of the absolute amount of each pigment. In order to lay an egg with relatively more protoporphyrin and less biliverdin, the mother must have the adequate stored resources to do so (Morales et al., 2011). If she gets these resources from her local food supply, and warmer temperatures can affect food supply, food quality and quantity may be reduced (Pearce-Higgins et al., 2010). Specifically, warmer temperatures can negatively impact the population size of common house sparrow prey, such as insects and other arthropods (Hanson et al., 2020; Pearce-Higgins et al., 2010).

As mentioned, I did not quantify pigmentation in terms of protoporphyrin and biliverdin content in this study, so the reason behind these color variations cannot be empirically validated. In some cases, it is possible that the visible coloration on an eggshell is not always indicative of the actual pigment concentration (Butler and Waite, 2016). In subsequent studies, it would be beneficial to sample the eggshells for their protoporphyrin and biliverdin levels in addition to conducting a color analysis to better explain the associations I found.

I hypothesized that the coloration and size in house sparrow eggs would be a result of the mother's condition just prior to and during egg-laying. I predicted that when eggs had more protoporphyrin, represented by red-shifted hues and lower brightness values, and were larger, the mother invested more into these eggs, signaling that she had greater fitness. This pattern has been identified in other species, as Great Tit (*Parus major*) females with higher hemoglobin levels laid larger eggs (Dufva, 1996). However, results have been mixed as to how the mother's condition can predict characteristics of her offspring. In pied flycatchers (*Ficedula hypoleuca),* for example, increasing ambient temperatures resulted in mothers laying larger eggs, but this occurred simultaneously to the mother's mass decreasing (Potti, 2008). This illustrates the trade-off between a mother and her offspring when reproducing: the more of her resources that she invests into her offspring, the less she has to put towards her own self-maintenance, such as her body mass and size (Potti, 2008). Since there are various mechanisms that can drive this trade-off, it is possible that some aspects of a mother's condition are more biologically relevant. Since ambient temperatures can have the potential to impact the mother, and, hence, her reproductive potential, these critical relationships will be more important as global warming progresses.

Shifts in ambient temperature as a result of climate change could also result in a mismatch between when environmental food supplies are optimal and when critical developmental stages occur (Sanz et al., 2003). For example, if annual temperatures begin to change earlier or later than usual, there could be a shift in when food quality and abundance is the highest. The breeding population may not be able to simultaneously shift their reproductive schedule to this change, leading to suboptimal food availability during the breeding season. As a result, it may be useful to explore how year-round temperatures affect local food supply, and not just the proposed implications of the food supply during the breeding season.

Hatching and fledging success

A larger proportion of eggs hatched when the historical temperatures were higher during the incubation period. Warmer ambient temperatures will cause the internal temperature of the nest cavity to simultaneously increase, and these warmer temperatures can shorten the length of the incubation period and allow the eggs to hatch earlier (Mueller et al., 2019). This relationship suggests that it may actually be advantageous for mothers to breed when ambient temperatures are warmer, as they would not have to incubate their eggs as long since the increasing temperatures are doing some of the work for them (Reid et al., 2000). By conserving energy during the incubation period, mothers can reallocate this energy to their offspring in the nestling period or towards other reproductive attempts during the breeding season (Reid et al., 2000). Cavity nesting species can also improve the insulation of their nests by utilizing nesting materials such as feathers and hairs, which are more effective at maintaining temperatures than sticks and other plant materials (Botero-Delgadillo et al., 2017). There would likely be an upper limit to what the internal nest cavity temperature could be before it starts to harm the eggs, but that determination would require more experimental manipulation.

Warmer temperatures also led to a greater proportion of nestlings successfully fledging during the nestling period. Depending on which covariates were in the model, temperatures that were warmer than the historical high or low temperatures were related to greater fledging success. Similar to the pre-laying period, during this time, the local food availability could likely impact offspring development. If warmer temperatures lead to a higher quality and quantity of resources, the parents will be able to provide better food for their young (Vatka et al., 2011). The parents also are responsible for keeping the nestlings’ body temperatures regulated, as the nestlings’ ability to do so would still be developing (Lowther and Cink, 2020; Węgrzyn, 2013).

Similar to the incubation period, warmer temperatures could increase the temperature inside of the nest, so the parents would not need to invest as much time or effort into keeping the nestlings warm. Overall, these results go against my predictions, as I hypothesized that warmer temperatures would decrease hatching and fledging success.

There are many possible explanations for these hatching and fledging results. First, house sparrows are non-native to Pennsylvania, which may affect how they react to changes in their environment. Beyond North America, house sparrows can be found in every continent except Antarctica (Hanson et al., 2020; Lowther and Cink, 2020), which all have very different weather patterns. Since they can survive in so many different climates, it is possible that they are better able to adapt to environmental changes than other native species. Species that are more accustomed to one specific area and its weather would likely not be able to adapt as quickly or as effectively as a species that is capable of living in so many different climates (Visser, 2008). Specifically, the plasticity of a house sparrow's digestive system allows it to not only adapt to a native diet, but also to adjust to more novel food options if resources become scarce (Hanson et al., 2020; Martin and Fitzgerald, 2005). Therefore, changes in temperature may not actually be as considerable of an obstacle to house sparrows and their behavior as I had predicted. House sparrows' ability to compete for, adapt to, and process a range of different foods may actually be a catalyst in their success as a non-native species as they can more easily adjust to changes in their environment. As a result, house sparrows may have more successful outcomes than other native species, even if they are all exposed to the same environmental conditions.

Another potential explanation for why house sparrows had greater developmental success in warmer temperatures could be because they are pre-adapted to do better in warmer temperatures. Native to the naturally-warm regions of northern Africa, the Middle East, and

Europe (Hanson et al., 2020; Sainz-Borgo et al., 2016), house sparrows have also established large populations in other warm climates, such as in Brazil (Wagner, 2012), Venezuela, Mexico, Australia, and the Caribbean Islands (Sainz-Borgo et al., 2016). With this expansion, more house sparrows have been drawn to more urban environments (Hanson et al., 2020), which are typically warmer than rural areas (Heinl et al., 2015). Factors that contribute to this warmth include higher levels of human activity, less vegetation, more industrial facilities, and greater population density (US EPA, 2014). Since house sparrows have demonstrated the plasticity to forage, develop, and compete effectively in their non-native, warm regions, selection may be favoring those offspring that are most successful in these regions.

In this project, I predicted that temperatures that were warmer, relative to historical temperatures, were above an optimal baseline for breeding season temperature, not the optimal level itself. When considering climate change, “warm” temperatures have been and will continue to get warmer. Average global temperatures have been steadily increasing for well-over a century, but the average rate of increase has more than doubled since 1981, at a rate of 0.18 degrees Celsius each decade (Lindsey and Dahlman, 2021). Further, as a result of climate change, increasingly warmer temperatures have been shown to have adverse effects on ecosystems, often targeting lower-level prey abundance (Pearce-Higgins et al., 2010) and plant reproductive success (Hedhly et al., 2009). As these factors make up the basis for house sparrow diet and nest-building, I predicted that the temperatures decreasing food and resource availability would act as a mechanism that would decrease breeding success for house sparrows. However, my data suggest that house sparrows may be more successful in a warmer world, giving them an advantage over other species that are not able to exploit newly available resources, or meet new environmental challenges.

Capital breeding versus income breeding

Based on the impact that pre-laying temperatures had on egg development, house sparrows are likely an income breeding opposed to a capital breeding species. For income breeding animals, temperatures during the pre-laying period have the potential to be a key determining factor in their reproductive efforts, as the temperature can affect the local food supply. Since income breeders collect most of their resources for reproduction in close proximity to when they begin breeding, the environmental conditions just before this would influence their investment into their offspring. Significant relationships were found between the pre-laying period temperature and egg size, eggshell brightness, and eggshell hue, and these correlations serve as evidence to support that house sparrows were income breeders. If they were capital breeding, the temperatures during the pre-laying period would not be very influential on their eggs' quality and appearance, as they would already have enough resources stored in their system that they would not need to rely on the current state of food availability.

There is also support that these house sparrows were income breeding based on their life history. House sparrows typically breed multiple times during the breeding season, and, in order to meet the demands of reproduction, they must continually replenish their resource stores (Lowther and Cink, 2020). Based on environmental changes that impact resource quality and abundance, house sparrows exhibit variation in offspring quality as a result. In addition, this ability to adapt during varying environmental conditions goes to support that house sparrows would be more successful as climate change progresses. Since I found support that income breeding is an advantageous strategy for house sparrows, and since they were more successful in warmer temperatures, climate change may favor a continued expansion of their population size. When competing in an environment with other species and limited resources, the ability to withstand and even thrive in warmer temperatures would be selected for as ambient temperatures continue to increase. While other species, namely capital breeding species, are naturally more affected by long-term changes in environmental resources, house sparrows can continue to succeed as income breeders in these fluctuating conditions.

Conclusions

Overall, this study supported the postulation that house sparrow reproduction is more successful in warmer temperatures. Specifically, warmer temperatures during the pre-laying period were associated with house sparrows laying larger eggs. Eggshell brightness was best predicted when the pre-laying temperature was greater than the mean historical temperature. In addition, eggshell hue was red-shifted as temperatures during the pre-laying period increased. These results were assumed to be linked to greater or lesser protoporphyrin and biliverdin content in the eggshells, but no relationships can be confirmed since I did not directly measure those pigments in my study.

A greater proportion of eggs hatched when temperatures were warmer during the incubation period. In addition, a greater proportion of nestlings fledged when temperatures were warmer than the mean historical temperatures during the nestling period. As global temperatures continue to rise, house sparrows will likely continue to successfully survive and breed, expanding their population all over the world and often as a non-native species. They likely will also be able to outcompete some other species, especially those that are not as equipped to adjust to long-term changes in temperature. While many studies have been conducted to analyze how changes in ambient temperature affect reproduction and developmental success, continued research will have to be done to continually add to this knowledge in the context of global climate change. In addition, since climate change is associated with a greater number of extreme weather events, other aspects of weather such as precipitation could be predictive of developmental success in house sparrows. Studying other weather conditions would allow for a more holistic view of how changes in climate impact animal development and what to expect for the ever-changing future of the planet. This path of study involving climate and developmental trade-offs is relevant now more than ever, and this expanding knowledge of the long-term environmental implications is only beginning.

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Tables and Figures

Table 1. Temperature metrics and abbreviations.

Temperature metric	Abbreviation
Mean temperature	T_{Avg}
Mean historical temperature	T_{HistAvg}
Mean difference between the mean and mean historical temperatures	T_{DiffAvg}
Mean maximum temperature	T_{AvgMax}
Maximum temperature	T_{Max}
Mean historical maximum temperature	$T_{\mathrm{HistAvgMax}}$
Mean difference between the mean and mean historical maximum temperatures	$T_{\mathrm{DiffAvgMax}}$
Maximum difference of all the differences of the mean and mean historical temperatures	T_{MaxDiff}
Mean minimum temperature	T_{AvgMin}
Minimum temperature	T_{Min}
Mean historical minimum temperature	$T_{\mathrm{HistAvgMin}}$
Mean difference between the mean and mean historical minimum temperatures	$T_{\mathrm{DiffAvgMin}}$
Minimum difference of all the differences of the mean and mean historical temperatures	T_{MinDiff}

Table 2. Repeatability (*r*) of egg appearance based on triplicate photos of the same egg.

Variable	*F*	Degrees of freedom	*p*-value	*r*
Maximum length	18.64	1268, 2592	< 0.0001	0.85
Maximum width	8.32	1268, 2592	< 0.0001	0.71
Volume	15.21	1268, 2592	< 0.0001	0.83
Ellipse deviation	22.37	1268, 2592	< 0.0001	0.88
Surface area	13.81	1268, 2592	< 0.0001	0.81
Hue	42.86	1232, 2463	< 0.0001	0.93
Brightness	69.45	1232, 2463	< 0.0001	0.96

Table 3. Repeatability (*r*) of multiple eggs within the same clutch, using average values for each egg.

Variable	*F*	Degrees of freedom	*p*-value	*r*
Egg mass	6.85	289, 997	< 0.0001	0.57
Average egg length	10.15	289, 997	< 0.0001	0.67
Average egg width	8.85	289, 997	< 0.0001	0.64
Average egg volume	10.84	289, 997	< 0.0001	0.69
Average ellipse deviation	5.72	289, 997	< 0.0001	0.52
Average surface area	10.64	289, 997	< 0.0001	0.68
Average egg hue	15.66	285, 965	< 0.0001	0.77
Average egg brightness	5.03	285, 965	< 0.0001	0.48
Average egg size	10.25	289, 997	< 0.0001	0.68

Table 4. Effect of pre-laying period temperature on egg appearance. All models within 2 of the lowest AIC value are listed, with the model with the lowest AIC value listed first.

Temperature variable in model	Dependent variables	*F*	Degrees of freedom	*p*-value	AIC
$T_{\text{HistAvgMax}}$	Average egg size	34.66	1,110	< 0.0001	1042.8
T_{HistAvg}		33.44	1,110	< 0.0001	1043.9
T_{DiffAvg}	Average eggshell brightness	18.39	1,108	< 0.0001	1716.6
$T_{\text{HistAvgMax}}$		17.96	1,108	< 0.0001	1717.2
T_{HistAvg}		16.66	1,108	< 0.0001	1718.5
T_{Max}	Average eggshell hue	18.23	1,108	< 0.0001	1892.8
$T_{\text{HistAvgMax}}$		18.64	1,108	< 0.0001	1892.9
T_{HistAvg}		17.46	1,108	< 0.0001	1894.0

Table 5. Effect of incubation period temperature on hatching success. All models within 2 of the lowest AIC value are listed, with the model with the lowest AIC value listed first.

Temperature variable in model	Covariates	*F*	Degrees of freedom	*p*-value	AIC
$T_{\mathrm{HistAvgMin}}$	Average eggshell brightness and average egg size in all models	4.30	1,104	0.0406	528.78
T_{AvgMax}		4.19	1,104	0.0432	528.91
T_{HistAvg}		4.12	1,104	0.0448	528.97
$T_{\mathrm{DiffAvgMin}}$		4.00	1,104	0.0481	529.09
$T_{\mathrm{HistAvgMax}}$		3.93	1,104	0.0500	529.17
T_{Avg}		3.12	1,104	0.0802	529.98
$T_{\mathrm{HistAvgMin}}$	Average eggshell hue and average egg size in all models	5.39	1,104	0.0223	526.67
T_{HistAvg}		5.22	1,104	0.0244	526.84
T_{AvgMax}		5.14	1,104	0.0254	526.91
$T_{\mathrm{HistAvgMax}}$		5.03	1,104	0.0270	527.03
$T_{\mathrm{DiffAvgMin}}$		5.00	1,104	0.0275	527.06
T_{Avg}		3.90	1,104	0.0509	528.18

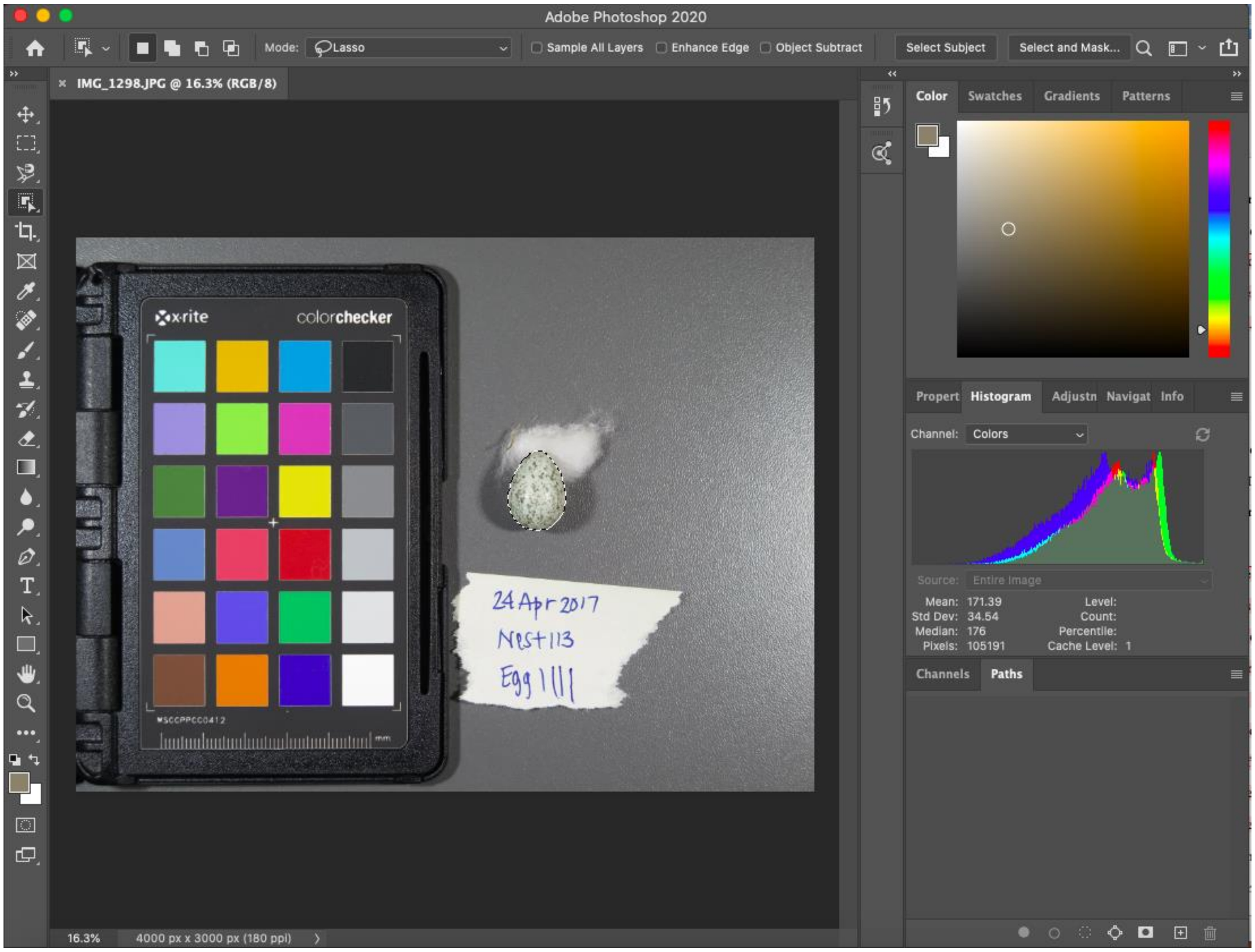
Table 6. Effect of nestling period temperature on fledging success. All models within 2 of the lowest AIC value are listed, with the model with the lowest AIC value listed first.

Temperature variable in model	Covariates	*F*	Degrees of freedom	*p*-value	AIC
$T_{\mathrm{DiffAvgMax}}$	Average eggshell brightness and average egg size	28.72	1,184	< 0.0001	472.36
$T_{\mathrm{DiffAvgMin}}$	Average eggshell hue and average egg size	28.53	1,184	< 0.0001	472.28

Developmental Event					
		Laying Begins	**Laying Ends** **Egg Photos Taken**	**Hatching Begins**	**Fledging Begins**
Day Range	**-------7-------**		**------------9----------**	**----------10---------**	
Temperature Stage	**Pre-laying Period**	**Laying Period**	**Incubation Period**	**Nestling Period**	

Figure 1. Timeline of developmental events for house sparrows. The pre-laying period begins seven days prior to when the first egg is laid. The laying period lasts from the day the first egg is laid until the day the last egg is laid. The incubation period begins on the day the last egg is laid and lasts for nine days. The nestling period begins on the first day of hatching and lasts for ten days.

Figure 2. Example of ImageJ set-up for determining egg size and shape. The outline was created from eight points using the Multi-point tool and then the Egg Measurement feature was used to create the yellow outline.



IMG_1298.JPG @ 16.3% (RGB/8)

Mode: Lasso

Channel: Colors

Source: Entire Image

Mean: 171.39
Std Dev: 34.54
Median: 176
Pixels: 105191
Level:
Count:
Percentile:
Cache Level: 1

16.3% 4000 px x 3000 px (180 ppi)

Figure 3. Example of Adobe Photoshop set-up for color analysis. The egg was outlined using the Object Selection tool, and this highlighted egg was then analyzed for hue and brightness using the Color Picker and Histogram features on the right.

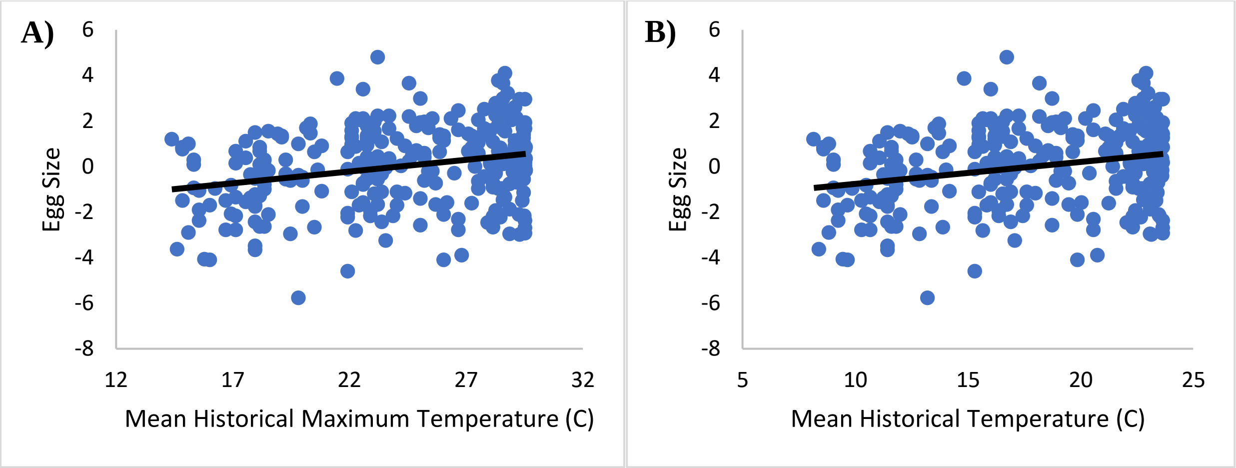


Figure 4. Effect of pre-laying temperature on average egg size in a clutch. **(A)** The average egg size in a clutch was significantly larger when the mean historical maximum temperatures were warmer during the pre-laying period ($F_{1,110} = 34.66$, $P < 0.0001$). This was the most predictive model for egg size. **(B)** The average egg size in a clutch was significantly larger when the mean historical temperature was warmer during the pre-laying period ($F_{1,110} = 33.44$, $P < 0.0001$).

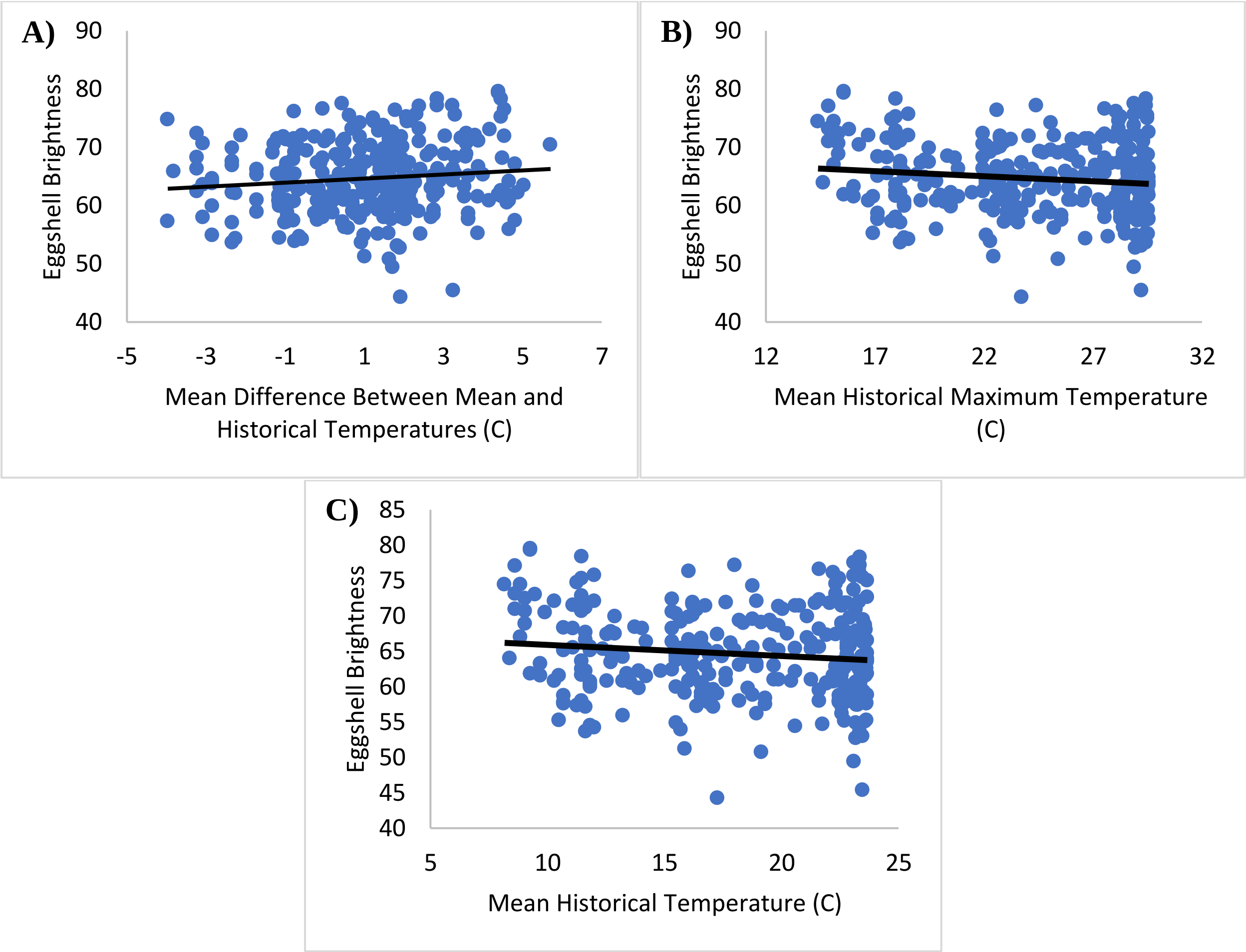


Figure 5. Effect of pre-laying temperature on average eggshell brightness in a clutch. **(A)** The average eggshell brightness in a clutch was significantly higher when the mean temperatures were warmer than the mean historical temperatures during the pre-laying period ($F_{1,108} = 18.39$, $P < 0.0001$). This was the most predictive model for eggshell brightness. **(B)** The average eggshell brightness in a clutch was significantly lower when the mean historical maximum temperatures were warmer during the pre-laying period ($F_{1,108} = 17.96$, $P < 0.0001$). **(C)** The average eggshell brightness in a clutch was significantly lower when the mean historical temperatures were warmer during the pre-laying period ($F_{1,108} = 16.66$, $P < 0.0001$).

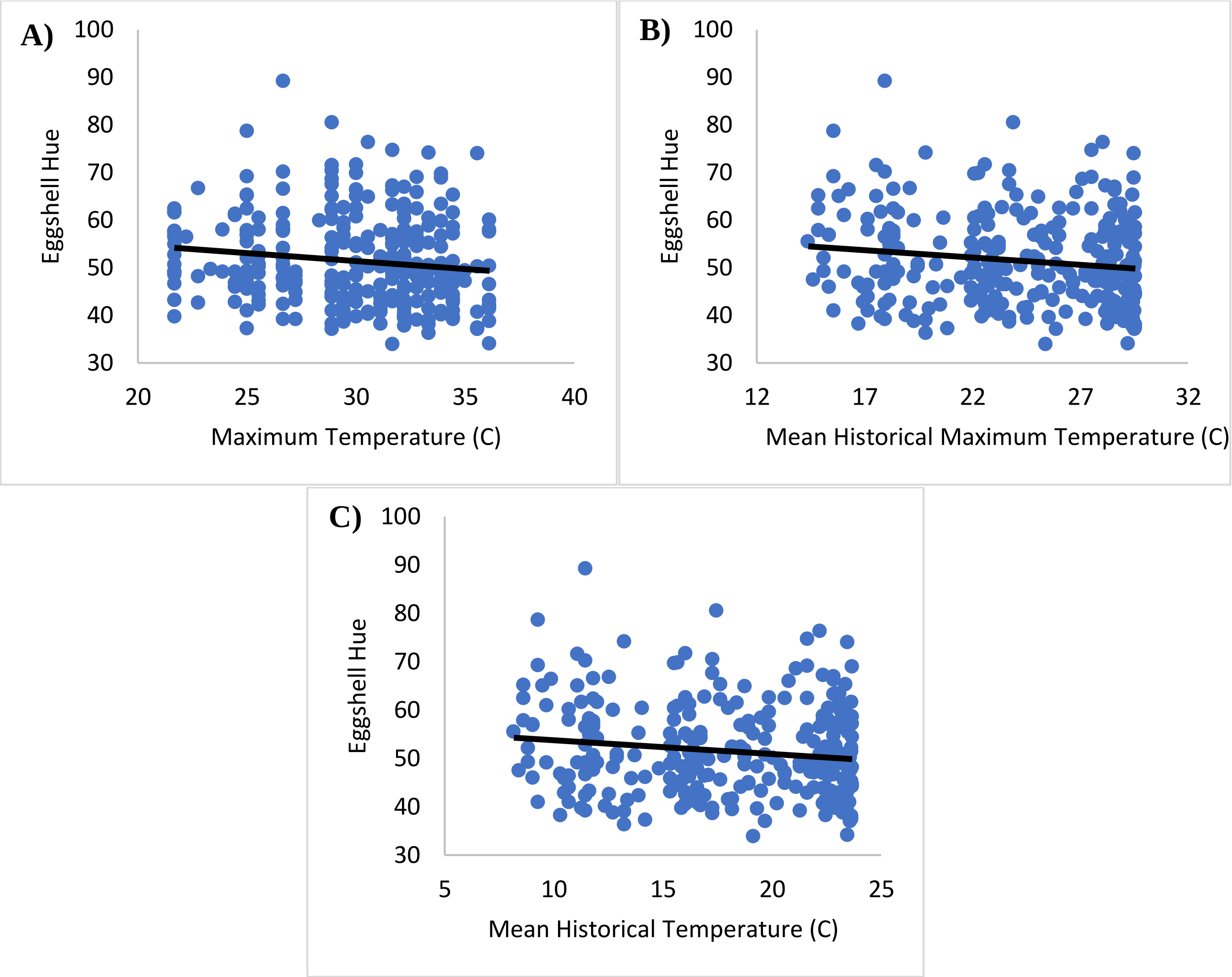
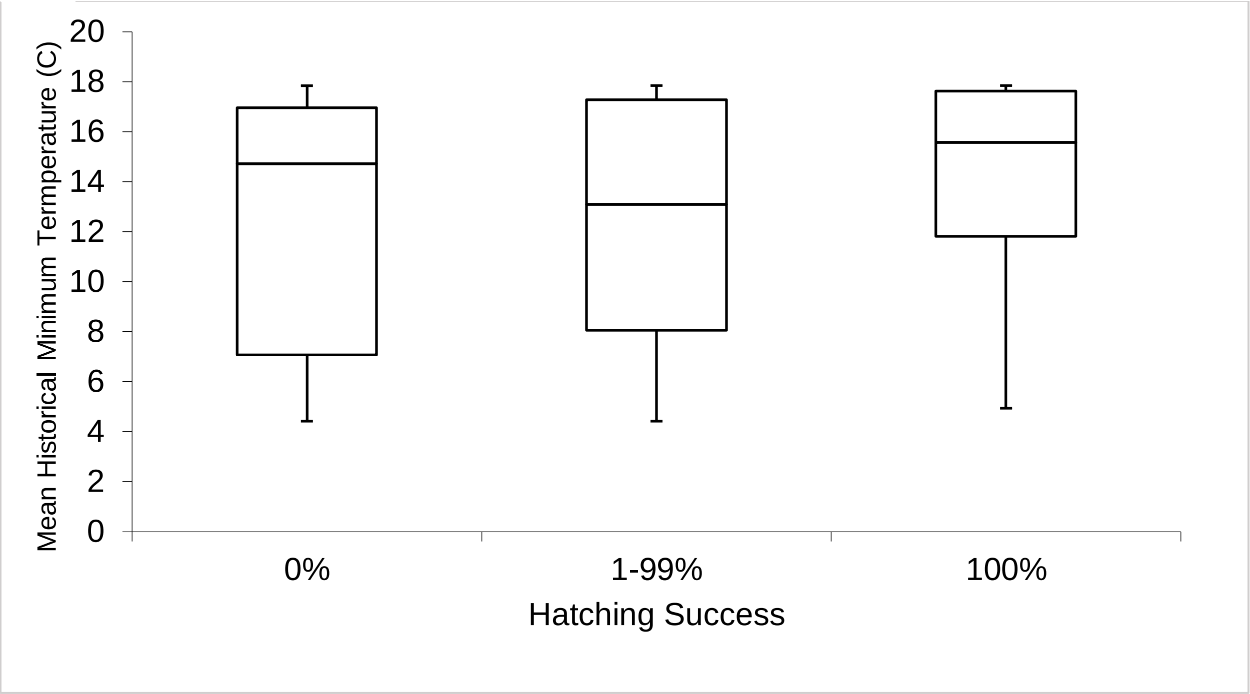
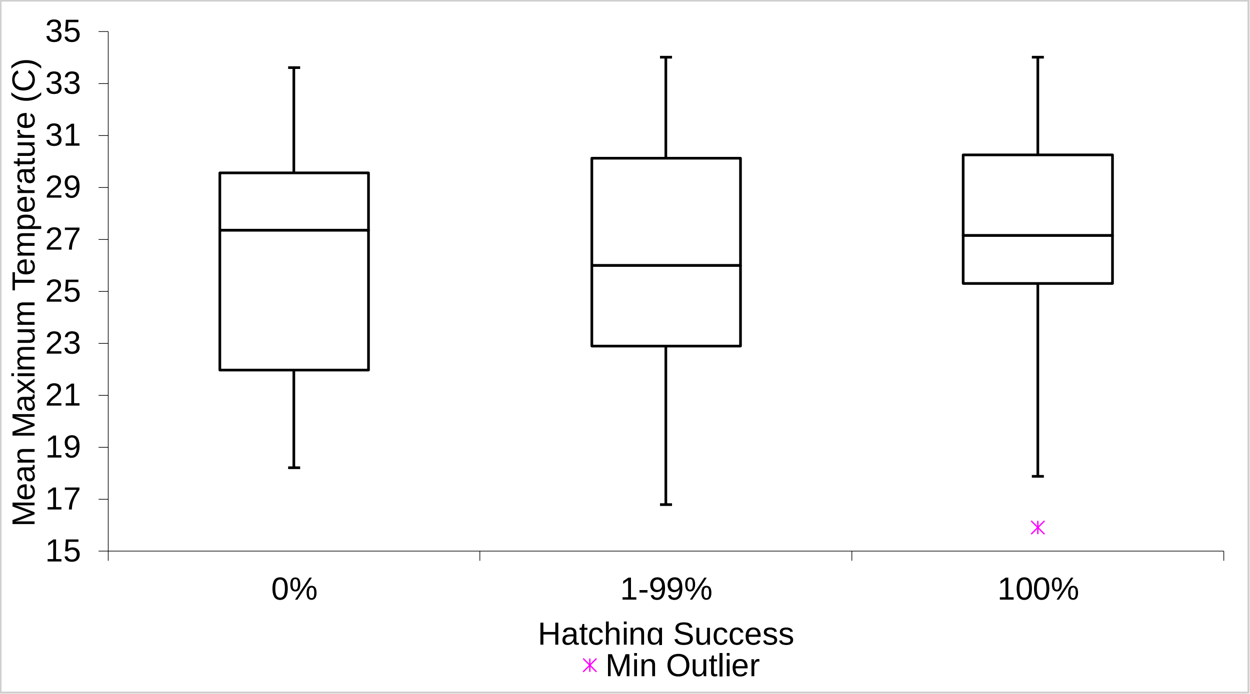


Figure 6. Effect of pre-laying temperature on average eggshell hue in a clutch. **(A)** The average eggshell hue in a clutch was significantly lower (red-shifted) when the maximum temperatures were warmer during the pre-laying period ($F_{1,108} = 18.23$, $P < 0.0001$). This was the most predictive model for eggshell hue. **(B)** The average eggshell hue in a clutch was significantly lower (red-shifted) when the mean historical maximum temperatures were warmer during the pre-laying period ($F_{1,108} = 18.64$, $P < 0.0001$). **(C)** The average eggshell hue in a clutch was significantly lower (red-shifted) when the mean historical temperatures were warmer during the pre-laying period ($F_{1,108} = 17.46$, $P < 0.0001$).

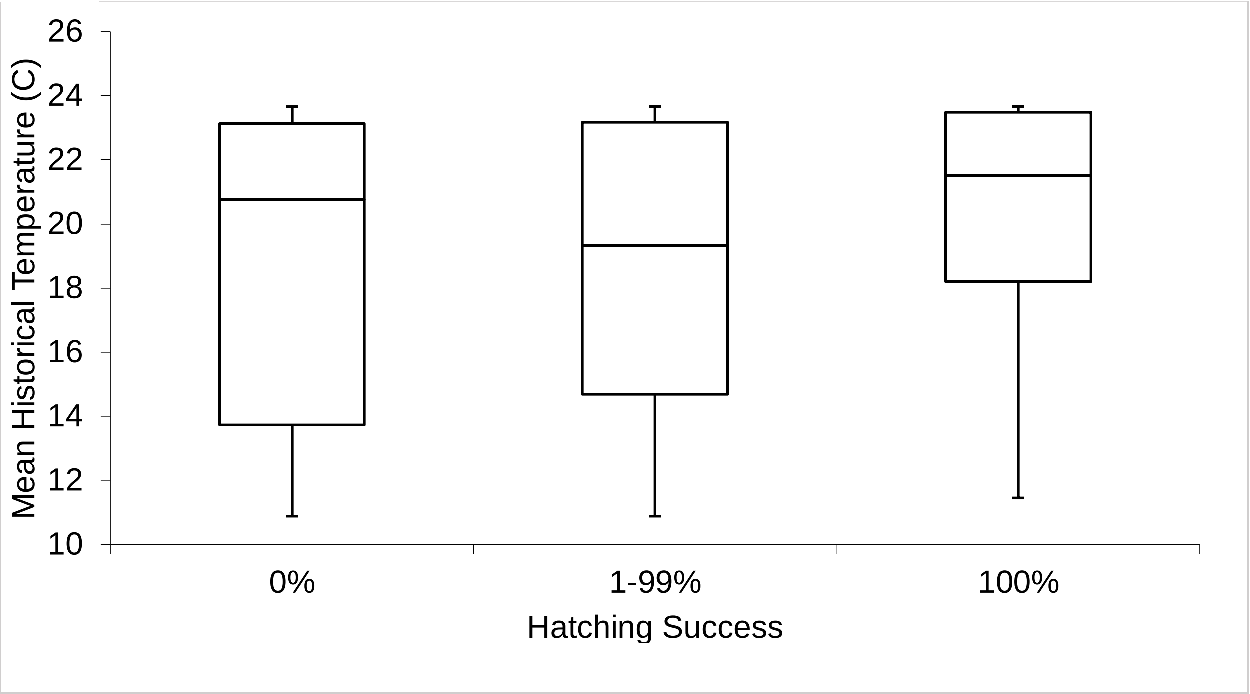
A)


Mean Historical Minimum Termperature (C)
0
2
4
6
8
10
12
14
16
18
20
0%
1-99%
100%
Hatching Success

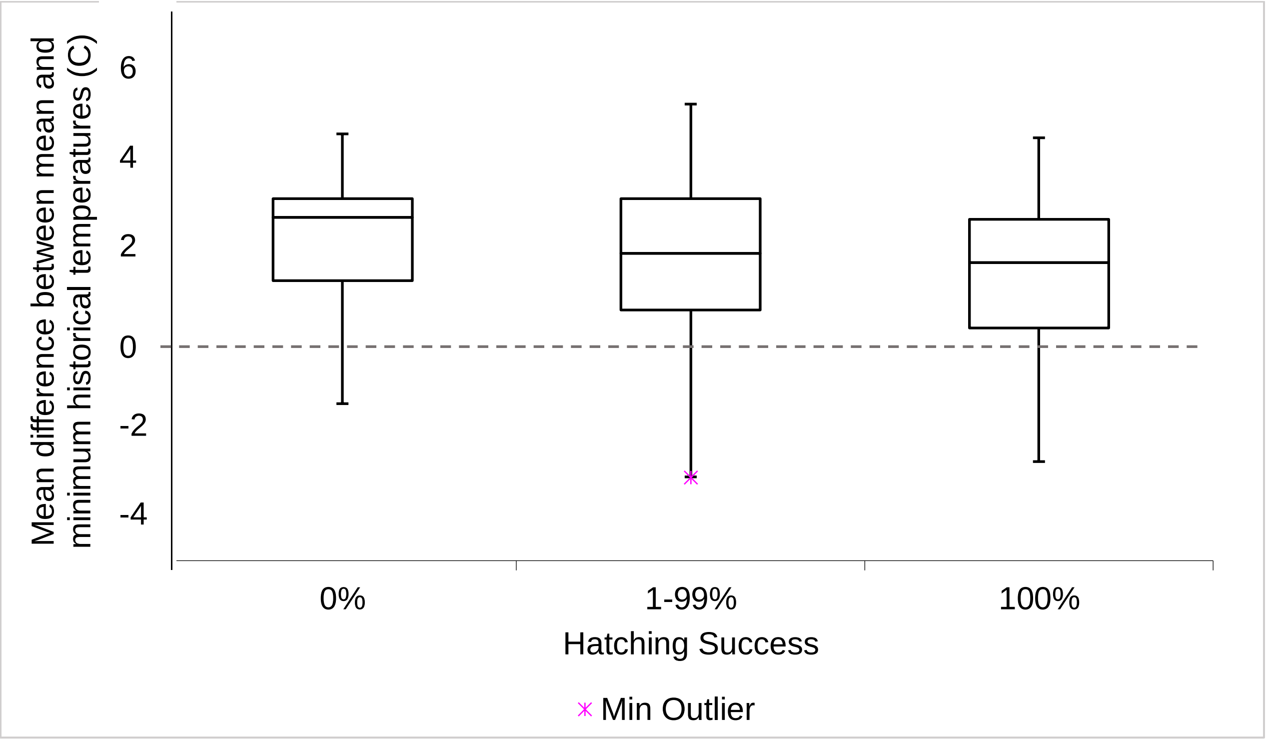
B)


Mean Maximum Temperature (C)
15
17
19
21
23
25
27
29
31
33
35
0%
1-99%
100%
Hatching Success
⁎ Min Outlier

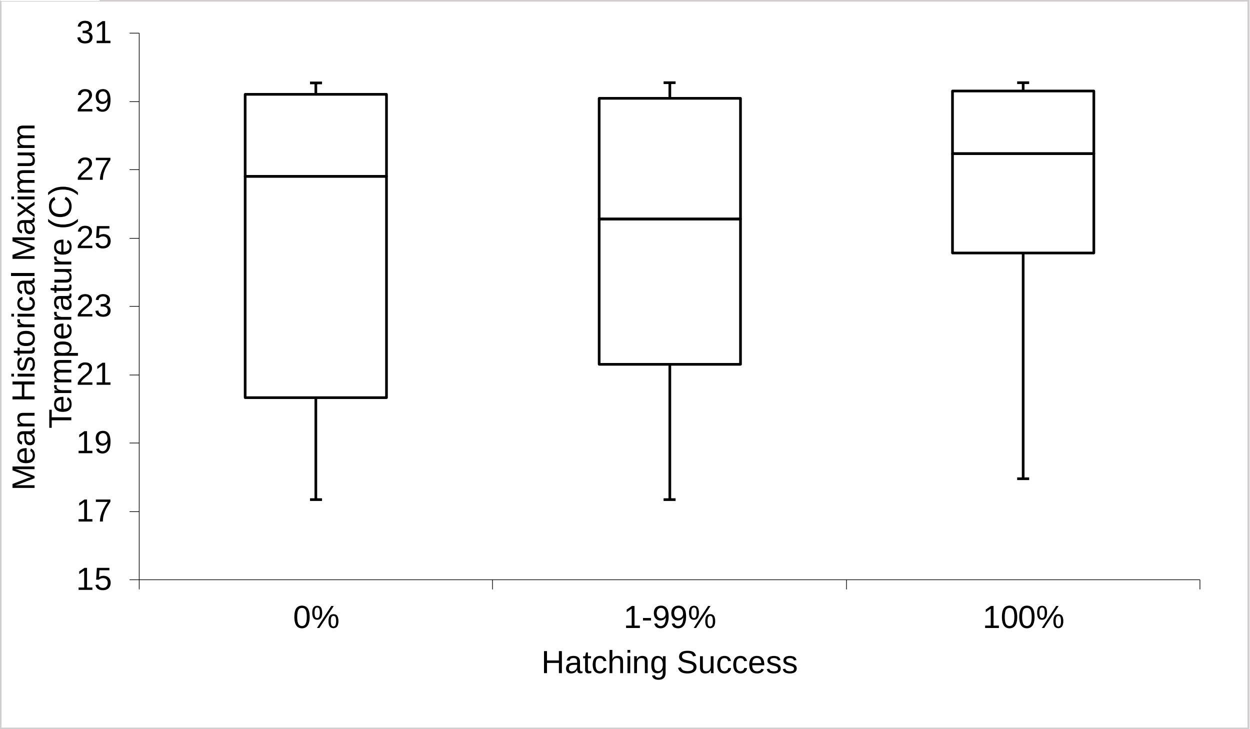
C)


Mean Historical Temperature (C)
26
24
22
20
18
16
14
12
10
0%
1-99%
100%
Hatching Success

D)


Mean difference between mean and minimum historical temperatures (C)
6
4
2
0
-2
-4
0%
1-99%
100%
Hatching Success
✳ Min Outlier

E)


31
29
27
25
23
21
19
17
15
Mean Historical Maximum Termperature (C)
0%
1-99%
100%
Hatching Success

F)

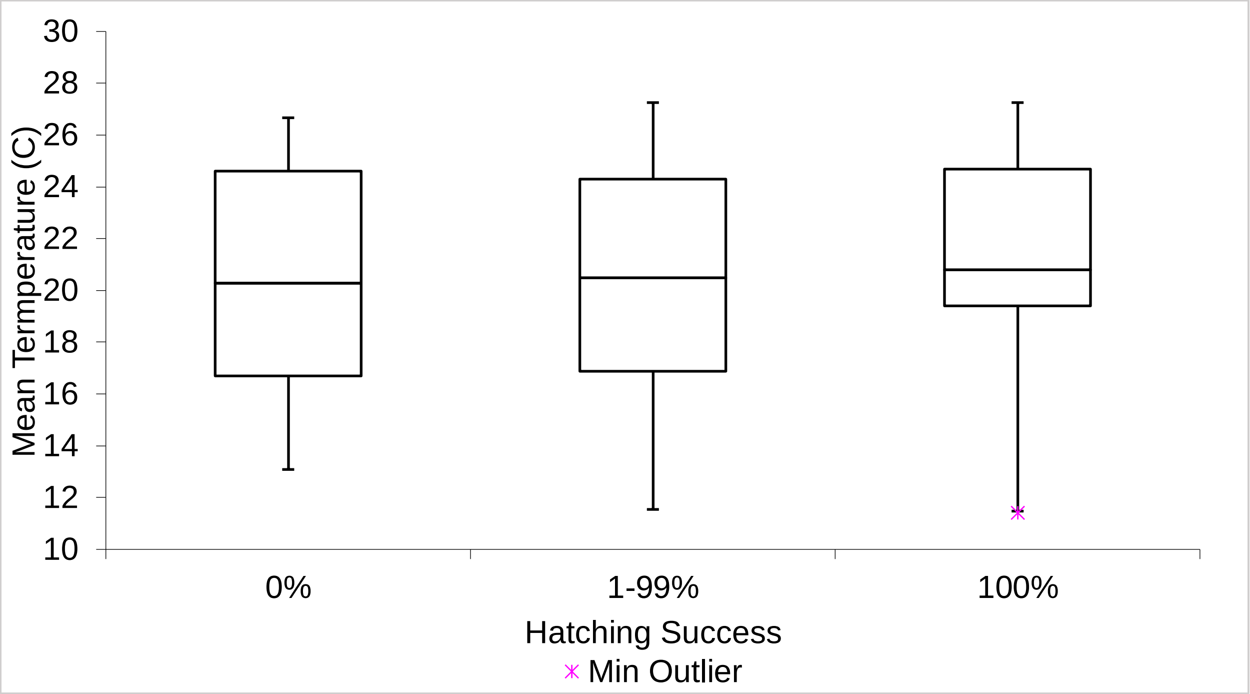
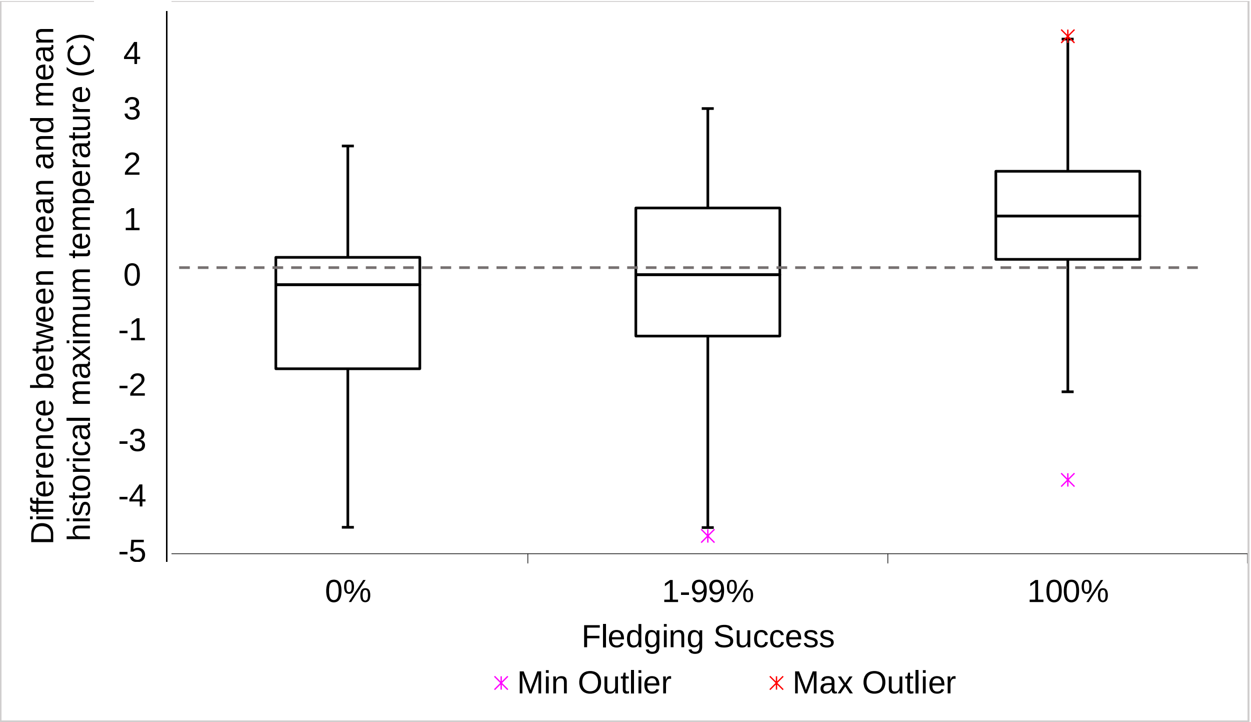

30
28
26
24
22
20
18
16
14
12
10
Mean Termperature (C)
0%
1-99%
100%
Hatching Success
✳ Min Outlier

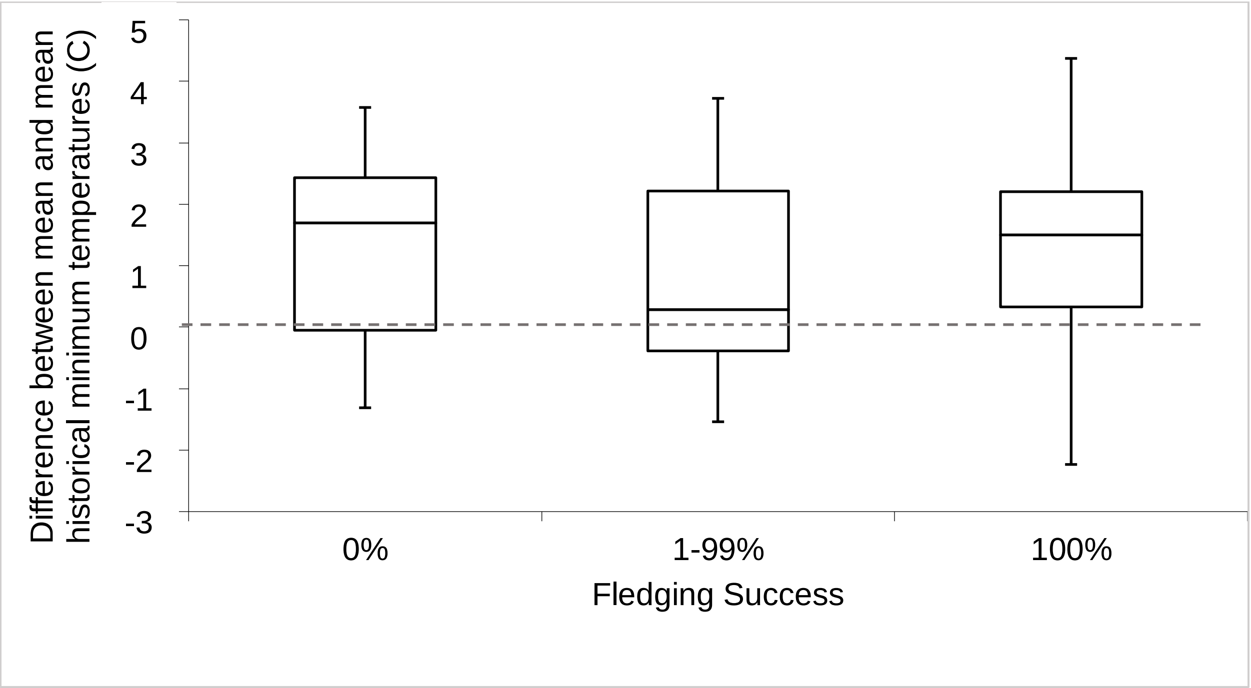
Figure 7. Effect of incubation period temperature on hatching success. Raw data are plotted, but statistical analyses accounted for covariates and random effects. **(A)** A significantly larger proportion of eggs hatched when the mean historical minimum temperature was warmer during the incubation period ($F_{1,104} = 4.30$, $P = 0.0406$). This was the most predictive model for hatching success. **(B)** A significantly lower proportion of eggs hatched when the mean minimum temperature was warmer than the mean historical temperature during the incubation period ($F_{1,104} = 4.00$, $P = 0.0481$). **(C)** A significantly larger proportion of eggs hatched when the mean maximum temperature was warmer during the incubation period ($F_{1,104} = 4.19$, $P = 0.0432$). **(D)** A significantly larger proportion of eggs hatched when the mean historical temperature was warmer during the incubation period ($F_{1,104} = 4.12$, $P = 0.0448$). **(E)** A significantly larger proportion of eggs hatched when the mean historical maximum temperature was warmer during the incubation period ($F_{1,104} = 3.93$, $P = 0.0500$). **(F)** There was no significant relationship between hatching success and the mean temperature during the incubation period ($F_{1,104} = 3.12$, $P = 0.0802$).

A)



Difference between mean and mean historical maximum temperature (C)
4
3
2
1
0
-1
-2
-3
-4
-5
0%
1-99%
100%
Fledging Success
⚹ Min Outlier
⚹ Max Outlier

B)



Difference between mean and mean historical minimum temperatures (C)
5
4
3
2
1
0
-1
-2
-3
0%
1-99%
100%
Fledging Success

Figure 8. Effect of nestling period temperature on fledging success. Raw data are plotted, but statistical analyses accounted for covariates and random effects. **(A)** A significantly larger proportion of nestlings fledged when the mean temperature was warmer than the mean historical maximum temperature during the nestling period ($F_{1,84} = 28.72$, $P < 0.0001$). **(B)** A significantly larger proportion of nestlings fledged when the mean temperature was warmer than the mean historical minimum temperature during the nestling period ($F_{1,84} = 28.53$, $P < 0.0001$).