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Analyzing avian incubation with a computer algorithm

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Abstract

A stable nest environment is necessary for incubation and development of offspring. Birds vary behaviour to regulate temperature for successful hatchlings. I used a hidden Markov model (HMM) to test how environmental conditions affect incubation behaviour. I examined nest temperatures and behaviours collected from Zebra finches that incubated at 30 °C or 14 °C, then incubated in the same or opposite condition for a second clutch. Data loggers and cameras recorded nest temperature and number of parents on nest. The HMM inferred behaviour from recorded behaviours and temperatures. Temperature and offspring success affected incubation duration. Birds that had successful offspring incubated at higher temperatures than unsuccessful birds. The HMM could not accurately predict number of birds from the temperature data because birds maintained nest temperature irrespective of behaviour. This study shows that birds can change behaviour in relation to the environment. Previous offspring success and ambient temperature are drivers of change in incubation behaviour.

Keywords

Summary for lay audience

Birds sit on nests to develop the embryos within the eggs they laid. However, the environment is not always good for embryo growth. One aspect of the environment which is important for embryo growth is the surrounding temperature. The birds have to change the way they sit on the nest because of the surrounding temperature. If the surrounding temperature is too warm or too cold, then the embryos may die or not develop correctly. I look at what birds do in these situations where the surrounding temperature is either cool or warm. I use a computer program I built to see if I can use a small sample of bird behaviour and nest temperature to predict how many birds are on the nest, given only the nest temperature. Zebra finches, birds that are native to Australia, laid eggs and sat on them in either 14 °C or 30 °C. The zebra finches then stayed in the same surrounding temperature or switched to the alternative for a second laying and sitting on eggs.

Birds need to consider the surrounding temperature, and their breeding experience at that temperature so that their chicks survive. I thought that if birds made mistakes in the first try in one of the surrounding temperature conditions, the second try in the same condition they would have more eggs hatched. If they were in a different condition for the second try, then they would not be as successful hatching eggs than if they were in the same condition. The surrounding temperature, and if the birds had hatched eggs had an effect on how long the birds needed to hatch an egg. Birds that had previously been successful hatching an egg had higher nest temperatures than unsuccessful pairs. My model was not able to predict behaviour from nest temperature. Birds can change how they sit on eggs to the surrounding temperature.

Co-Authorship Statement

I conducted all statistical analyses described in the thesis, wrote and tested the hidden Markov model, and am responsible for all interpretation and conclusions presented in the thesis. Jeff Martin of Western University, Sophie Edwards of the University of St. Andrews, and I collected all of the data described in the thesis as part of a large-scale joint project on nest building behaviour and incubation with Dr. Sue Healy of the University of St. Andrews and Dr. David Sherry of Western University. Some of these data were used in my undergraduate thesis. Jeff Martin and I conceived the experimental design and outlined the methodologies to be used. Sophie Edwards was responsible for all video recordings. Results on nest building behaviour and nest structure will be published elsewhere. Dr. Mark Daley provided expertise and guidance in writing and testing Hidden Markov Models.

Erich R Eberts and team at the Loyola Marymount University, through the LMU Center for Urban Resilience was the group credited for the Allen's hummingbird data set. Erich is responsible for all experimental design, data collection, and processing of the Allen's hummingbird data set.

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Dr. Mark Daley took this project, again that was out of his research field. However, his insight and compassion towards my learning process was always timely. I may never be able to shake the feeling of not having the skills to do the ideas I have, but Mark would always uplift me and remind me of how far I have come.

Jeff Martin has been a steadfast mentor and colleague throughout this project, as a sounding board for analyses and interpretations, as well as someone to empathize with over the data. This project would not be as robust, successful and interesting if it was not for Jeff's dedication and critical thinking. Thank you for keeping my wild conclusions in check.

Dr. Ben Rubin, much thanks goes to for his advice on which statistical tests to run and comprehending the results. Dr. Rubin as well I would like to thank for giving me the confidence to pursue mathematics, by admitting mistakes and working through problems together made me realize everyone makes mistakes and that equations are only useful if they help understanding.

I would like to thank Adriana Diez for her teachings on how best to care for zebra finches and sharing her experience from over the years. As well, all the volunteers that cared for the zebra finches; caring for 72 zebra finches is no small feat. Thank you Erich Eberts for the hummingbird data set, without it I would be forever wondering if I had only encoded more data maybe the model would work… This project would not have been possible without the numerous users of Stack Overflow and Github, whose replies aided me in my time of programming struggle.

Lastly, I would like to extend all my gratitude to my husband, Phil. He has listened to all my presentations, looked over codes, read over papers, and learned more than I am sure he cares to have ever learned about zebra finches. What sanity I have left is because of you.

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Chapter 1: Introduction

1 Avian incubation and mathematical models

Incubation is the period during avian reproduction when birds sit on the eggs to keep a stable temperature until the eggs hatch. It is during this time that the embryo inside the egg develops into a chick. Fluctuating environmental conditions (i.e., temperature, precipitation, and wind) can put pressure on birds to change how they incubate to maintain a stable temperature and microclimate for their eggs (Love, Gilchrist, & Semeniuk, 2010; Ninnes et al., 2011).

Avian incubation is how birds can maintain the nest environment for suitable embryo development. Birds achieve the ideal nest environment by careful control of temperature, humidity, and gas exchange. As with most physiological processes, embryo development requires a narrow range of temperatures (35 °C -38 °C) for successful hatching. The bird maintains this nest temperature by sitting on the eggs, however the length of time sitting on the nest and how the nest is built are factors that lead to successful offspring (Deeming, 2002). If the nest temperature becomes too cool (less than 30 °C), birds have been observed shivering and feather raising or fluffing up; whereas if the nest temperature becomes too warm (greater than 40 °C) the birds open their mouths to release heat (gular fluttering) (Deeming, 2002).

As one or both parent birds are required to be on the nest to maintain a satisfactory temperature, the other tasks such as foraging for food and grooming each other must be modified. To achieve this, most birds have both parents incubating like the zebra finch (*Taeniopygia guttata*). There are exceptions though, with some species having only one bird incubate, whether it is the female such as in the Allen's hummingbird (*Selasphorus sasin*) or the male. The amount of time spent on the nest varies widely across species whether it is constant sitting on the nest or sporadic incubation sessions. Nest temperature and number of birds on the nest are part of the equation that can lead to successful offspring. The next section (1.1) will delve into how birds cope when environmental conditions are challenging.

1.1 The effect of environmental cues on avian incubation

Environmental variables such as precipitation, wind, temperature and elevation are challenges for birds during reproduction (Heenan, Goodman, & White, 2015). Yet, as birds are found from the rainforest to the Antarctic, they have evolved diverse behavioural mechanisms to adapt to seemingly inadequate reproductive environments. A general mechanism that birds use is to time their reproduction to food availability (Love, Gilchrist, & Semeniuk, 2010; Ninnes et al., 2011; Stouffer, Johnson, & Bierregaard Jr., 2013). Near the equator, birds generally breed throughout the year with some relation with rainfall, as food is plentiful. In the temperate regions, birds generally breed in conjunction with the seasons, as food resources are scarce during the winter (Stouffer, Johnson, & Bierregaard Jr., 2013). This example of birds adapting to environmental conditions is a gross difference between the temperate and equatorial regions. Can birds show similar changes to behaviour when environmental conditions change rapidly?

The Adélie penguin (*Pygoscelis adeliae*) has been seen to exhibit rapid behavioural change in line with the ice sheets receding. Adelie penguins modulate their incubation duration due to the amount of ice sheet still present to allow for the best chance of chick survival from one year to the next. The differences between incubation duration is observed not only from year to year, but also compared to different colonies, where ice melt differs (Ninnes et al., 2011). Shortening the incubation period to coincide with food availability is one method of behavioural change to adapt to environmental conditions (Ninnes et al., 2011).

The common eider (*Somateria mollissima*) takes a slightly different approach when faced with changing environmental conditions. Instead of changing its number of days during incubation, the common eider changes its lay date, but maintains the same number of days for incubation. The behavioural change is correlated with both the ambient temperature and ice melt, to time hatching with the receding ice and increased food availability (Love, Gilchrist, & Semeniuk, 2010). Changing lay date and incubation duration are two mechanisms birds have employed to synchronize reproduction to changing environmental conditions, and thus food availability.

Not all birds, however, show this relationship between environmental change, and subsequent reproductive behavioural change. Rhinoceros auklets (*Cerorhinca monocerata*) were observed under variable field conditions for four years, with no significant change in incubation following environmental fluctuation and widely variable breeding success (Hipfner, Mcfarlane-tranquilla, & Addison, 2008). The success of offspring did not influence the next reproductive attempt. Another example of a bird species unable to change their reproductive behaviour to changing environmental conditions is the great tit (*Parus major*), who after 23 years of study has not changed its lay date to accommodate the increased ambient temperature. The great tit is at a disadvantage as it is missing potential food resources before its clutch has hatched (Visser, Noordwijk, & Tinbergen, 1998).

The research into how birds can change their incubation behaviour to react to changing environmental conditions has shown potential mechanisms, from changing incubation duration to changing the lay date of the eggs to coincide with available food resources. All the included studies have taken place in the field, so the ecological validity of the findings is high. An understanding of which environmental conditions are cuing these behavioural changes is difficult to establish. My project aims to recreate reproductive conditions while in the lab. The lab allows for a higher number and precision of measurements. Investigating incubation within a controlled lab setting will provide an opportunity to look at a single environmental variable and its effect on incubation.

1.2 Nest-building and incubation behaviour, two methods to cope with the physiological limits of egg development

Incubation duration and egg lay date need to align with food availability, but also the physiological requirements needed for embryo development. Eggs require a narrow range of temperatures to reach hatching with proper development. Approximately 35 °C must be maintained for egg viability. Any consistent temperatures below the 35 °C threshold will halt development of the embryo, or lead to lower rates of survival as the offspring matures (Berntsen & Bech, 2015; Durant, Hepp, Moore, Hopkins, & Hopkins, 2010; Durant, Hopkins, Hepp, & Walters, 2013; Wada et al., 2015; White & Kinney,

1974). For example, Wada et al., (2015) found that even 1 °C change from the ideal temperature can lead to poorer offspring development and survival.

There are two potential methods to maintain 35 °C, either: 1) the birds can construct a nest that will keep heat from interspersed incubation bouts, or 2) incubate for an amount of time that maintains the required temperature with the nest retaining little heat. It is possible that birds use some combination of both (Deeming, 2002).

In theory, the more insulation a nest has, the less the parents must incubate to achieve the ideal incubating temperature. However, previous literature has found little evidence to support the theory that more insulation is used to offset incubation duration. When researchers studied the structural and thermal components of cup-shaped nests, they found that nest shape was for structural support of the eggs and parents rather than for insulation (Heenan & Seymour, 2011).

An example illustrating that modulating nesting material may not be the method used to control nest temperature is arctic birds. One would assume an extreme amount of nest insulation would be needed, however, nests did not have a higher amount of insulation—if any—than would be expected for the climate (Irving & Krog, 1956). Because of these studies, it can be concluded that modulating nesting material may not be a strong method for maintaining nest temperature.

Instead of changing nest shape for insulation, birds can alter incubation duration as a fine scale adjustment of the nest microclimate (Deeming, 2002). A pair could incubate in 15 minute increments with two birds on the nest always, then after 15 minutes both birds leave the nest; whereas another pair may have one bird incubating constantly, presuming the birds switch off seamlessly. Incubation duration will likely influence the amount of time required to hatch, as seen in the Adelie penguin example (section 1.1).

Birds can change how long they sit on the nest to regulate temperature, but also can adjust the heat output they produce. Previous research has shown that if the number of eggs is increased, female zebra finches (*Taeniopygia guttata*) will increase their heat output (Hill, Lindström, Mccafferty, & Nager, 2014). As well, at more variable temperatures, the male plover (*Charadrius spp.*)*,* increases his incubation temperature output (Alrashidi et al., 2016). The female zebra finch and male plover are both able to modulate their heat output to keep their eggs at the right temperature.

Changing incubation behaviour is a response to the environmental conditions. The more experience a bird has in the environment, the more it will learn what modifications are required during reproduction for successful offspring. Birds have been shown to integrate previous experience into their preferences during reproduction. Experience with nesting material or environmental factors have been shown to elicit rapid plastic behavioural responses in birds. For example, experience can lead to sensitivity to colour, rigidity of material, precipitation or wind direction, and birds will change their nest-building behaviour to withstand these environmental pressures (Muth, Steele, & Healy, 2013; Muth & Healy, 2011; Bailey, Morgan, Bertin, Meddle, & Healy, 2014; Heenan, 2013). Taken what is known about birds' ability to modulate nest temperature and their behavioural flexibility, I aim to delve further into when and how birds change their incubation behaviour.

In both section 1.1, and the current section 1.2, numerous examples of birds adapting to environmental cues have been put forward. These studies use manual data collection where there is a possibility for subjective and erroneous recordings. My project improved the previous research by recording data in a controlled lab environment to look at one environmental cue— temperature— and by automating a part of data collection to increase objectivity, while decreasing human error.

This MSc project investigated when and what kind of changes in incubation behaviour occur. I examined a large data set consisting of automatically recorded nest temperatures and video recordings of parental nest attentiveness that I had previously collaborated in collecting as part of a large-scale project on nest building behaviour in zebra finches. In this thesis, I examine the effect of ambient temperature and previous incubation experience on nesting success in zebra finches. I develop a Hidden Markov model (HMM) and apply it to the time series of recorded nest temperatures and parental nest attentiveness by zebra finches, and also apply this model to another smaller dataset of Allen's hummingbird incubation behaviour obtained by thermal imaging.

1.3 Zebra finch incubation

Zebra finches (*Taeniopygia guttata*) were chosen to study when and what kinds of changes in incubation behaviour occur with ambient temperature change. They are

opportunistic breeders and thus breed throughout the year. Therefore, their ability to change behaviour according to differing environmental temperatures should be evident. Zebra finches are extensively studied; their incubation behaviour and physiology are well described (Nord, Sandell, & Nilsson, 2010; Salvante, Walzem, & Williams, 2007; Zann & Rossetto, 2014).

To understand how zebra finches change their incubation behaviour, the typical reproductive behaviours are first described. In zebra finches, the breeding cycle begins with the male and female completing a courtship routine, with the male singing. The male then builds a nest. A typical zebra finch nest is constructed in a dome shape. The dome shape can include a roof, though not all zebra finch nests do. The female contributes little to building of the nest. After internal insemination, a clutch of eggs is laid (Morris, 1954). The female takes on the primary role of incubating the eggs, though the male will aid in incubation (Gorman & Nager, 2003; Hill, Lindström, Mccafferty, & Nager, 2014; Zann & Rossetto, 2014). The average percent of total time spent on the nest during incubation is 91.1% +/- 10.8% for Passeriformes who share incubation of altricial young, which zebra finches fall into (Deeming, 2002). Each clutch consists of approximately 4-6 eggs, with the female only able to lay one egg each day (Griffiths & Gilby, 2013). The nest-building takes roughly 10 days, followed by an incubation of 14 days and then fledging for another 14 days. After the hatchlings have fledged from the nest, they can live independent from their parents (Morris, 1954).

1.4 Hummingbird incubation

As small birds, hummingbirds face an even greater challenge of keeping an optimal thermal climate in the nest as compared to other birds. However, Calder (1971) observed Calliope hummingbirds (*Stellula calliope*) that reproduce at high elevation throughout the Cascade, Sierra and Rocky Mountains and when compared to Anna's hummingbirds (*Calypte anna*) that breed at lower elevations, found no difference in the number of days taken to incubate. Calder's finding that high elevation hummingbirds need no more time than low elevation hummingbirds illustrates that the high elevation hummingbirds must be using one or more of the adaptive mechanisms detailed before—

whether it is optimizing the type of nest, proportion of the days sitting on the nest, or the heat output that the hummingbird is expelling.

As hummingbirds do exhibit behavioural changes to environmental stimuli, they will be used as a comparison to the zebra finches, as only the female hummingbird incubates the eggs, thus creating an even more challenging situation in terms of energy expenditure and offspring survival. For my project, I was able to use a data set collected by Erich Eberts of the Allen's hummingbird (*Selasphorus sasin*). The Allen's hummingbird female creates a nest using downy materials for the interior and grass and leaves for the exterior, spider webs are used as an adhesive. The clutch size is two eggs, with the second egg being laid two days after the first. Multiple clutches are laid throughout the breeding season. Only the female incubates for 15 days, with the attendance increasing after the laying of the second egg. The female is on the nest constantly, save for feeding. Females will change their position on the nest to change nest temperature (Clark & Mitchell, 2013). Having only one parent possible to be on the nest at one time allows for a simpler data set to test the model on.

1.5 Hidden Markov models

1.5.1 Basic components of a hidden Markov model (HMM)

Hidden Markov models are used to analyze time series data consisting of states and observations. Let us consider the example of a diligent graduate student working in a windowless lab. She would like to know what the weather is outside. In this example, the weather can only be sunny or rainy. An assumption of the HMM is that only the previous day influences today's weather. These states follow the Markov property, of being dependent on just the previous state (**Equation 1**). The current state is required to make predictions about the next state, but the rest of the past states are completely irrelevant.

$$\Pr(C_{t+1} \mid C_t \dots C_1) = \Pr(C_{t+1} \mid C_t)$$

Equation 1. The Markov property. The history of state C from the initial state C_1, to the present state Ct predicts the next state C_{t+1} with the same probability as using the most recent value of C_t. That is, the next state depends only on the current state.

In this example, the weather is the state, and there are only two possible states that follow one after the other: sunny or rainy. There is an initial or starting probability of it raining or being sunny. As days go past, the weather has the possibility of changing from sunny to rainy, rainy to sunny or for it to remain sunny or rainy. These transitions from one weather state to the next have associated probabilities—transition probabilities—of the likelihood that one weather state would follow the previous one. This is visualized in the top portion of **Figure 1** (Zucchini & MacDonald, 2009).

However, as the name suggests, these states are hidden in this hidden Markov model. The only method to discern the weather is from the shoes of people passing by the lab, because the lab has no windows. The weather state is the cause of the footwear observations, but the studious graduate student cannot directly see the weather. In this lab, people generally wear rain boots when it is raining, running shoes when it is sunny and cowboy boots irrespective of weather conditions. However, fashion trends have created a world that it is not a certainty that all people wearing rainboots means it is raining outside, as shown in **Figure 1**. The relationship between footwear and weather can be used to determine the weather, as knowing that most people wore rain boots and cowboy boots would mean it is most likely raining. Thus, even though the state is hidden, it can be inferred from direct observations.

In my project, the states are the number of birds on the nest: 0,1, or 2 birds on the nest. The observations are nest temperature. As nest temperature is related to the number of birds on the nest, the hypothesis that I am testing with the HMM is that by knowing what the temperature is at a given point, it is possible to determine the number of birds on the nest at that point.
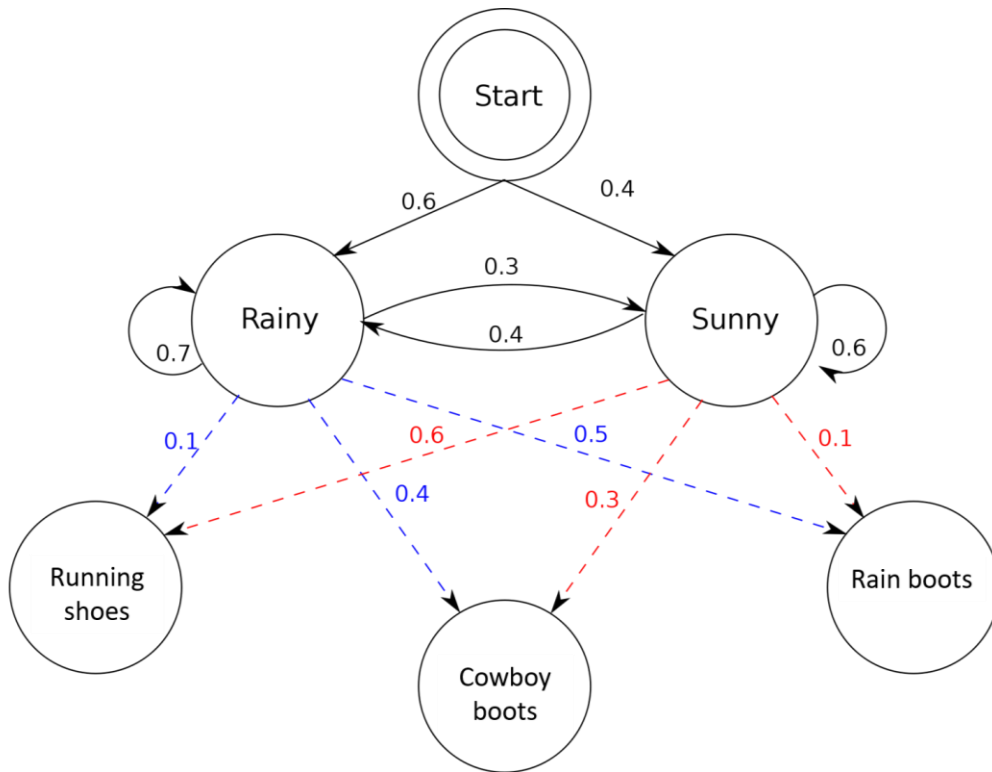
Figure 1. Example of a HMM application. The states of rainy and sunny have a starting probability of .6 and .4 respectively. Given that it is rainy, there is a .3 chance it will become sunny and a .7 chance it will continue raining. Given that it is sunny, there is a .4 chance that it will start to rain, and a .6 chance that it will continue to be sunny. There is a relationship with footwear worn by individuals and the weather, where given that it is rainy there is a .1 chance people will wear running shoes, .4 chance people will wear cowboy boots and a .5 chance people will wear rain boots. The same can be done for when it is sunny, there is a .6 chance of people wearing running shoes, .3 chance people are wearing cowboy boots, and .1 chance people are wearing rain boots. This relationship between footwear and weather can be used to predict the weather, given the footwear is known.

1.5.2 Decoding states from observations

The HMM takes a subset of recorded behaviour states and temperatures, creates starting and transition probabilities from that training set of known states and temperatures, and applies those starting and transition probabilities to a new set called the test set of temperatures only, where the model predicts the most likely state given the temperature. The training set is the subset of behaviour states and temperatures that the HMM generates probabilities from, and bases all behaviour predictions upon. Therefore, the training set should be a representative sample of the data set, otherwise probabilities and predictions would not be accurate. How I generate a training set is included in section 2.2. The values for temperature that occur in the test set list of temperatures must be values that occur in the training set, as only temperatures present in the training set will have probabilities associated with them. For example, if the case that 32 °C as an observation does not occur in the training set, but does occur in the test set, there will be no associated prediction as to the state at that temperature. Although all the values for temperature in the test set must be present in the training set, no particular sequence of temperatures has to be, as the HMM only relies on the previous state to generate predictions.

The rest of this section delves into how the transition and starting probabilities are generated.

This section follows Zucchini and MacDonald (2009), primarily their section 5.3.1. The main problem that the HMM aims to address for the current project is the ability to inform the researcher for any given temperature in the training set, what is the most likely behavioural state. To be able to calculate the most likely state from temperatures, forward and backward probabilities are required. The forward probability is calculated by taking the first observations in the sequence and finding the likelihood of being in a specific state. The backward probabilities instead assume a state and calculate the probability of obtaining future observations. The forward and backward probabilities can be used in conjunction to predict any state for any specific time, however, they cannot predict the most likely sequence of states (**Equation 2**).

$$\alpha_t(\text{i})\beta_t(\text{i}) = \Pr(X^{(T)} = x^{(T)}, C_t = i).$$

Equation 2. Forwards (α) and backwards (β) probabilities for each of the possible states i, is equal to the probability of one observation for the most recent time point ($x^{(T)}$) being equal to one out of the possible observations $X^{(T)}$, given that the state is one (C_t) out of the possible i.

For every time point (t), one can predict the most likely state C_t, given the observations $x^{(T)}$. For the current problem, *global decoding* is required as the sequence of behaviour states $c_1, c_2, c_3 \ldots c_T$ is sought. As forward and backwards probabilities can only give the most likely state at a given time, and the sequence of most likely states is required, the Viterbi algorithm is used (Viterbi, 1967; Forney, 1973; see Zucchini & MacDonald, 2009 for the proof).

1.6 HMMs in animal behavior

For my project, I used a HMM to infer behaviour states from nest temperatures. Previous work has shown how successful HMMs can be at discerning "hidden" states from observations. Past uses have included human speech recognition, transmembrane protein topology, and brain imaging (Krogh, Larsson, Heijne, & Sonnhammer, 2001; Rabiner, 1989; Zhang, Brady, & Smith, 2001). Within ecology, a similar approach to what I have taken has been abundant: an animal behaviour that is not directly observable is inferred from collected data. Dean et al. (2012) used a HMM to understand behavioural differences between two colonies of seabirds (*Puffinus puffinus*). The researchers used ground speed from GPS recordings and saltwater immersion to train the HMM to predict which of the three states, foraging, flying and sitting the seabirds were most likely in. The HMM was then trained, and able to use just the GPS recording and saltwater immersion data to accurately predict which behavioural state the seabirds were in. The result from the model was that the two colonies of birds foraged in two generally exclusive areas, with one small area where both colonies foraged together.

Franke, Caelli and Hudson (2004) used a HMM to validate previously collected caribou (*Rangifer tarandus*) movement data. The three states of interest were bedding, feeding, and relocating. The observations to infer these states were distance between locations and turning angle, from GPS collars. The HMM trained on the GPS data was able to accurately predict which behavioural state the caribou were in. Those HMM state predictions were then compared to auto-regressive model predictions, with the former being more accurate (Franke, Caelli, & Hudson, 2004).

Schliehe-Diecks, Kappeler, and Langrock (2012) used a slightly more sophisticated HMM to address individual differences. The HMM was used to infer motivational states, specifically hungry or satiated in grey mouse lemurs (*Microcebus murinus*). The observations were sex, body mass and time of night. The observations were used to infer motivational states (hungry/satiated), however there were extensive individual differences between the grey mouse lemurs. These differences were encapsulated by adding random effects—akin to random effects in a regression model—to their HMM. These random effects controlled for individual differences. The HMM with random effects allowed for insightful conclusions about grey lemur appetitive states, such that they change states much more often than the authors hypothesized in conjunction with time of night (Schliehe-Diecks, Kappeler, & Langrock, 2012).

HMMs have been used to understand complex systems when measured in the field, as illustrated here. For each of these examples, the behavioural states have discrete and mutually exclusive signatures within time and space—like in the caribou example the caribou cannot be both moving and sleeping, and the GPS data has a specific signature that defines moving from sleeping. In the current project, incubation posed a challenge to the HMM as detecting one or two birds on the nest may be a state change not as closely linked to the observations than the previous literature shown here.

1.7 Hypothesis and predictions

I hypothesized that ambient temperature, and the birds' experiences at that ambient temperature affect how birds incubate. I predicted that at a low ambient temperature, parents spend more time incubating to maintain the 35°C necessary for

development. This is accomplished by an increase in continuous incubation or shorter trips away from the nest. The opposite would be expected at a high ambient temperature. I also predict that birds with more experience at a certain temperature will use their experience to inform the subsequent breeding attempt by either doing the same incubation behaviour if they were successful, or adjusting their incubation duration to be different than before.

To test this hypothesis, I observed 36 pairs of zebra finches at different temperatures at the known ambient temperature limit of the breeding range. I collected nest temperature recordings from time the first egg was laid to the first hatchling hatched. 18 pairs were then either kept in the same ambient temperature condition for a second breeding cycle while 18 pairs were switched to the alternate ambient temperature condition, to address the aspect in my hypothesis about temperature-related experience. The same nest temperature was recorded for Trial 2, along with behavioural video recordings. The duration of incubation in days, hatchling success, and number of eggs was recorded for Trial 1 and 2. A subset of behaviours during incubation in Trial 2 were encoded, along with the respective nest temperatures. The subset of behaviour and nest temperature was used to train the HMM. The HMM predicted behaviour states from nest temperatures, which allowed for detailed analyses of incubation behaviour, and the differences that arose between ambient temperature conditions. Further method detail can be found in Chapter 2.

In Chapter 3 I present results on number of eggs and successful hatchlings per ambient temperature condition. The differences in incubation duration per ambient temperature and breeding experience are shown. The distributions of nest temperatures for 30 °C and 14 °C are compared, along with how the distributions shift when comparing successful and unsuccessful pairs. Accuracy of HMM for predicting behaviour from temperature is calculated for both the zebra finch and hummingbird data set.

Chapter 2: Methods

2.1 Zebra finch incubation

2.1.1 Subjects and husbandry

Thirty-six male-female pairs of sexually mature zebra finches were used in this study. All zebra finches were purchased from pet stores. The zebra finches were all given one breeding attempt prior to the start of the experience to ensure all pairs had at least one breeding experience to partially factor out lack of breeding experience. This breeding attempt was halted before egg hatching so no one pair had successful breeding that others did not. Throughout the study, birds had *ad libitum* access to water, mixed seeds, cuttlebone, and oyster shell grit, along with two tablespoons per pair of eggmix per day. Eggmix contained boiled egg with shell, corn meal and bread, blended using a food processor. Birds were given water dishes for bathing on a weekly basis.

Pairs were housed in individual cages (46 cm x 47 cm x 46 cm). Isolation walls that obstructed the pair's view of the neighbouring pairs were affixed to the cage with zip-ties. These isolation walls were composed of white Bristol board and cut to fit the three sides of each individual cage. Cages were arranged in the room to prevent pairs from viewing other pairs. For the second trial, black Bristol board was used on the back wall for added contrast when viewing the video recordings. Each cage contained a food cup, grit cup, water bottle, cuttle bone and a nest cup which was a plastic tray generally used under small plant pots (89 mm in diameter) The tray was hot glued to a U-shaped bolt affixed to the cage.

Two climate-controlled chambers were used to house the two ambient temperature conditions separately. Each chamber was temperature controlled ($\pm$ 0.1 °C) while providing approximately 15 air exchanges per hour (15 % fresh air, 85% recirculated HEPA filtered air). The chambers were 2.8 m wide x 2.85 m long x 2.3 m high.

Both conditions were on a 14:10 hr light: dark schedule, with full spectrum lighting and initial temperature range of 19-22 °C, 50-70 % humidity. Temperature was adjusted by 1.5 °C every 12 hrs, until the desired temperature 14 or 30 °C, was reached.

Plastic nest cups were supplied to each pair along with naturally uncoloured Everlasto flexible cotton string, with a diameter for 2.5 mm (James Lever & Sons Ropes & Twines, England, UK). Each pair started with 300 pieces of string (15 cm in length). Once the pair had used the original 300 pieces, they were given an additional 100 pieces. This continued until they did not use the string or four days went by, whichever came first. Four days was used as a standard nest-building period and the number of days where string was provided was not increased as to discourage nest rebuilding.

2.1.2 Procedure

Both Trial 1 and Trial 2 were completed as part of my undergraduate thesis project, where only the nest-building phase of reproduction was of interest. For my Masters degree data regarding the incubation phase of reproduction was used.

2.1.2.1 First breeding trial

This experiment investigated how zebra finch pairs adapted their incubation behaviour to different temperatures. The pairs were randomly assigned to either the 14 °C or 30 °C condition. Each condition contained 18 pairs. Maxim iButtons DS1925 +/- 0.5 °C temperature loggers, were used to measure temperature in the nest. An iButton was placed beside the first egg laid and set to record temperature every five minutes. This was done only for pairs that had kept their egg in the nest for 24 hrs. Pairs were monitored every other day to reduce intrusion on the nest. Number of eggs laid was recorded as well as if any eggs were discarded by the pair. Eggs laid outside of the nest cup were discarded by the experimenter, unless three successive eggs were laid outside the nest cup, then the next eggs would be left. The iButton logged data until the parents were taken out of the condition, along with any hatchlings. For the purpose of this experiment, only the period between the laying and hatching of the first egg was considered, to avoid temperature interference from the hatchlings.

2.1.2.2   Second breeding trial

To examine the effect of temperature-related experience on incubation behaviour, a second breeding trial was completed. After the first breeding trial, temperatures were systematically changed by 1.5 °C every 6 hrs until 22 °C for both conditions was reached.

The pairs had at minimum one week between breeding trials at 22 °C. This week was in a separate room than the environmental chambers to control for any noise effects in the chambers. Then at the start of the second breeding trial, the birds were placed in the appropriate environmental chamber and temperatures were decreased or increased by 1.5 °C every 12 hrs in the same method as Trial 1.

The same zebra finch pairs were used for the second trial, half of the pairs from the 14 °C chamber in the first trial were switched to the 30 °C chamber, and half from the 30 °C condition were switched to the 14 °C chamber. The other half of the pairs in each temperature remained in the same temperature condition for the second trial. Breeding success in Trial 1 was counter-balanced across conditions for Trial 2. As there were five pairs in each of the original 14 and 30 °C conditions that successfully raised hatchlings to fledge, those 10 pairs were spread randomly across each of the four conditions in Trial 2. This counter-balancing ensured there was no unequal distribution of previous hatchling success across the Trial 2 conditions.

There were 9 pairs in each of the four second trial conditions: 14 °C to 14 °C, 14 °C to 30 °C, 30 °C to 30 °C and 30 °C to 14 °C. Once the pairs were given string for nesting material, video cameras were set up to record the top two rows of the three rows of cages in each cage rack. Six out of the 17 cages for the 30 °C condition, eight of the 15 for the 14 °C were not manually encoded due to the constraints of the video cameras used. One pair in the 30 °C, and two pairs in the 14 °C were not used as one of the individuals in the pair had died prior to the start of Trial 2. The videos made it possible to view each nest, and whether there was a male or female zebra finch on the nest. Video records were collected until the pairs and hatchlings were removed from the conditions, at approximately four weeks. IButtons were placed as per the first breeding trial. Pairs were monitored every day for number of eggs laid and any eggs that were discarded by the pair. If the pair laid eggs in anything other the nest cup, the eggs were discarded by the experimenter, if three successive eggs were laid in anything other than the nest cup, they were left. After the iButtons were collected from the nests, the data were analyzed with Python 3.6., R programming language, and Microsoft Excel.

2.2 HMM implementation

2.2.1 Extracting data from iButton and inputting into HMM

The purpose of the HMM was to derive incubation behaviour from the iButton temperature data. Three pairs per condition were picked that had the best video quality. One day of behavioural states, male (M), female (F), both (B) or neither (N) bird on the nest, was manually encoded into Microsoft Excel. These behavioural states were synchronized with their respective iButton temperature recordings. The synchronization was performed by matching the iButton serial number associated with a pair to the pair's video recording. Then, the time stamp on the iButton was compared to the video recording, so that the behavioural states matched the exact time the iButton recorded temperatures. I later discarded the sex categorization as the model was unable to account for the differences between sex, and simplified the states to 0,1,or 2 birds on the nest.

I then had a 483 x 2 matrix of behavioural states and temperatures for the 30 °C condition, and a 429 x 2 matrix of behavioural states and temperatures for the 14 °C condition. The different rows between conditions was due to randomization of start time, as some start times for manually encoding behaviour included time when the cage was dark. The rows in the matrices (483 for 30 °C, 429 for 14 °C) were each time point; the first column was the nest temperature and the second column was the behaviour state. I ran an auto-regressive moving average (ARMA) regression with condition for trial 2 (30 °C or 14 °C) and behaviour (M,F,B,N) as independent variables, with pair as a random effect, and temperature as a dependent variable. I ran the regression to investigate whether there were any differences between pairs in the same ambient temperature condition. As the only significant term was ambient temperature condition ($t_{108} = 9.7$, p < .001), I increased the length of the time series by concatenating pairs into one long time series for each ambient temperature condition.

The first record for the second pair followed the last point of the first pair and, the first time point for the third pair followed the last time point of the second pair, separately for ambient temperature condition. These two matrices were used as training sets for the HMM, the number of behaviours per pair are shown in **Figure 2**. As detailed in section

17

1.5.2, the training set is used to generate the starting distribution and transition probabilities for the HMM.

I input either the 14 °C or 30 °C training set, with a test set of nest temperature data only. The HMM outputs a string of states that it predicts are the most probable, given the training set, the previous state, and the temperature at the current and previous time.

To validate these predictions, I manually encoded a validation set for each condition. A validation set consisted of one hour of manually encoded behavioural states per pair. For pairs that were included in the training set, an hour that was not included in the training set was encoded. These validation sets were 141 x 2 and 83 x 2 matrices of temperature and behaviour for 30 °C and 14 °C, respectively. The different row numbers for each condition are due to less cages in the 14 °C condition having video clear enough to manually encode (11 pairs used for 30 ℃ and 7 for 14 °C validation sets). Only the column containing temperature was input into the HMM as a test set. The resulting predictions were compared to the manually encoded behaviours and given a score of 0 or 1, 0 being incorrect prediction, 1 being correct prediction. The scores were tallied and the proportion of correct predictions to total predictions was calculated.
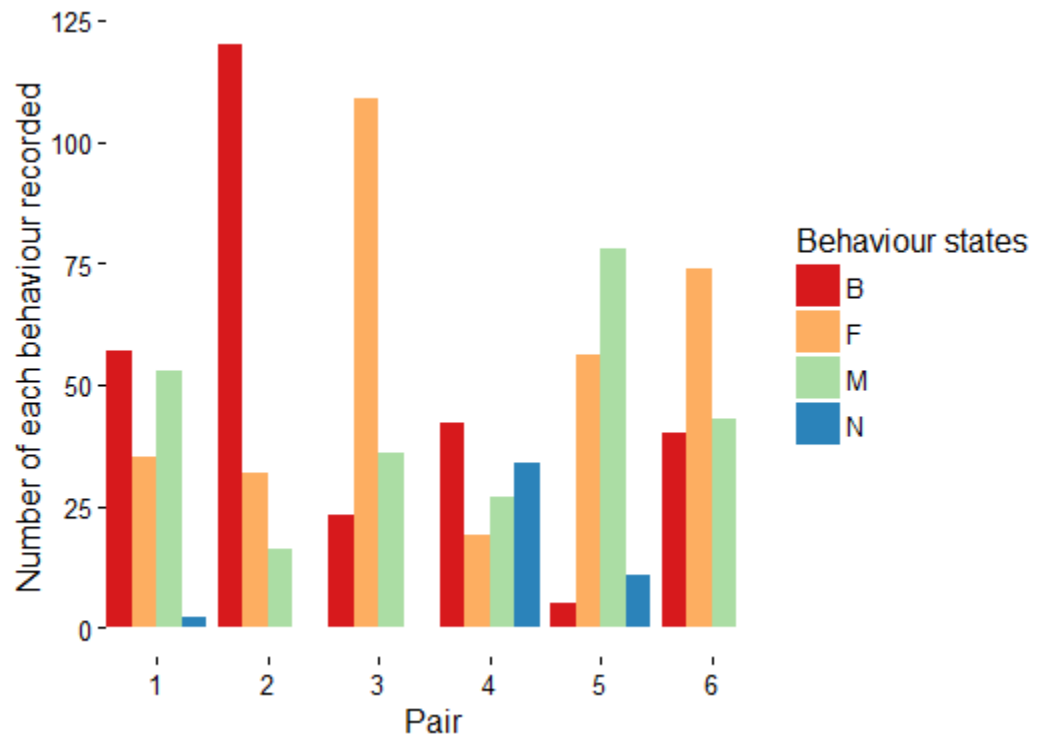
Figure 2. Number of behaviour states for the training set. Pairs 1 to 3 were from the 30°C condition, pairs 4 to 6 were in the 14°C condition. M in blue are male, F in green are female, N in purple are neither on nest, B in red are both on nest. Pairs were concatenated together (where the end of pair 1 is directly followed by the start of pair 2, and so on) to generate a suitably long sequence of both behaviour and temperature data for three pairs.

2.2.2 Data analysis

The success of pairs in each ambient temperature condition was compared to see if ambient temperature influenced the number of days required for incubation, and the proportion of successful pairs. All statistics were completed with an alpha value of .05. A Mann-Whitney test was used to assess number of days as the data was not normal, and a chi-square test of proportions was used to assess the proportion of pairs in each developmental stage (no eggs/eggs/hatchlings). This was completed separately for Trial 1 and Trial 2. To test the effect of temperature-related experience (Trial 1 temperature and Trial 2 temperature) along with the effect of breeding success in Trial 1 together on the number of days required to hatch was combined in a 2x2x2 ANOVA.

To understand the differences between successful and unsuccessful pairs I plotted the distributions of nest temperatures separately for successful and unsuccessful pairs, and observed the differences. I did the same for the two ambient temperature conditions. I evaluated the HMM behaviour predictions using accuracy scores generated from the validation sets, as detailed in the last paragraph of section 2.2.1. I compared the behaviour state proportions between the training and test sets, as well as during different times in incubation. I used the hummingbird dataset for further testing of the HMM accuracy using the same method as the zebra finch dataset.
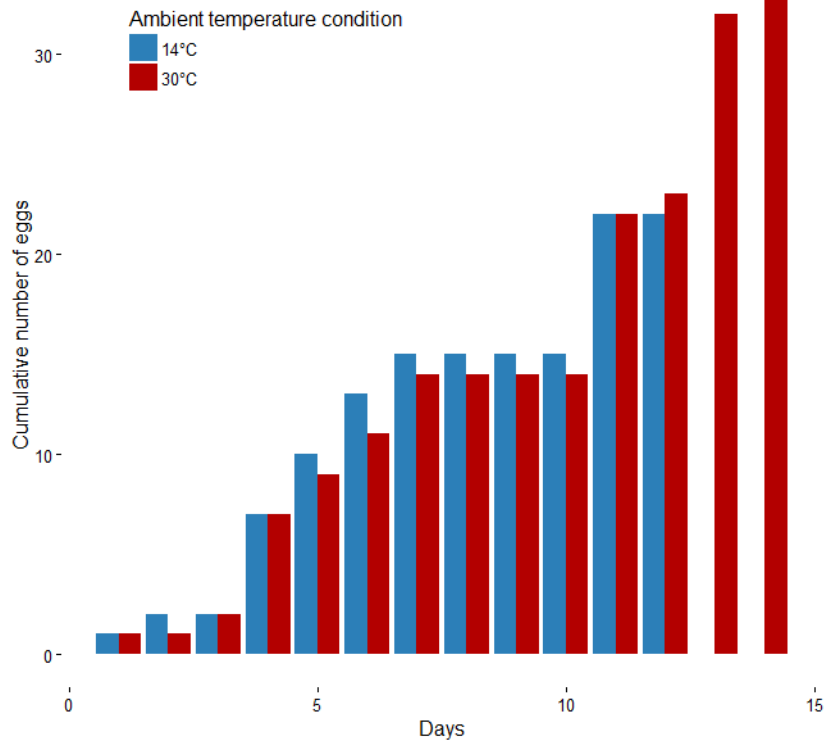
Figure 3. Cumulative number of eggs recorded per condition for all pairs in Trial 1, 30 °C shown in red with a sample of 16 pairs, 14 °C shown in blue with a sample of 12 pairs. Days as recorded by first egg laid, the same day for both conditions. The number of eggs recorded are only shown prior to first hatchling. The 14 °C condition hatched earlier than the 30 °C, which is why the red bars continue further than the blue. The total number of pairs with successful hatchlings was significantly higher in the 30 °C condition than the 14 °C condition ($\chi^2_1 = 4.2$, p = .04).

Chapter 3: Results

3.1 Zebra finch incubation

3.1.1 Trial 1

Eight pairs out of 34 had no eggs, (2 out of 17 in 30 °C condition), 14 pairs produced eggs without hatchlings (6 in 30 °C condition), and 14 (10 in 30 °C condition) produced hatchlings during the experiment (**Table 1**). I did not run statistical tests on absolute number of eggs laid, as some nests were difficult to enter without disturbing the pair. **Figure 3** shows the cumulative number of eggs laid and should be taken as an approximation of absolute eggs laid. The cumulative number of eggs was calculated by adding eggs recorded to a tally per pair. This led to egg counts always increasing or plateauing as time went on, even if the absolute number of eggs decreased. The cumulative tally was then summed with the rest of the pairs in each respective ambient temperature condition. The cumulative number of eggs laid between the two ambient temperature condition is similar (**Figure 3**). The 30 °C condition had significantly more hatchlings than the 14 °C condition, as tested by a chi-square test of proportions (χ^2_1 = 4.2, p = .04). The 30 °C condition incubated significantly longer than the 14 °C condition, as tested with a Mann-Whitney test (**Figure 4**, first column) (z = -3.41, p = .0003). Including only pairs that successfully hatched eggs, the trend of the pairs in the 30 °C condition taking more days to incubate persists (**Figure 4**). There were significantly more pairs in the 30 °C condition with hatchlings than the 14 °C condition, and those pairs needed significantly more time to incubate in Trial 1.

3.1.2 Trial 2

Two pairs had no eggs, (two in 30 °C condition), nine pairs produced eggs without hatchlings (two in 30°C condition), and 23 (13 in 30 °C condition) produced hatchlings during the experiment. No difference in number of successful hatchlings was found between the two ambient temperature conditions for Trial 2, as tested by a chi-square test of proportions ($\chi^2 1$ = 1.2, p = .27) see **Table 1**.

Trial 2 had significantly more pairs that had successful hatchlings, as tested by a chi-square test of proportions ($\chi^2 1 = 5.8$, p = .02). The number of incubation days to hatch in Trial 2 showed the reverse trend that Trial 1 showed, with 14 °C pairs taking significantly longer than 30 °C pairs to hatch an egg, as tested by a Mann-Whitney test (**Figure 4**, second column, **Figure 5**) (z = -1.61, p = .05).

To test the effects of ambient temperature and breeding experience on hatching success, I ran an 2x2x2 ANOVA with Trial 1condition (30 °C or 14 °C), Trial 2 (30 °C or 14 °C), and hatchling success in Trial 1 (yes or no) as fixed effects, with an interaction between temperature condition in Trial 1 and temperature condition in Trial 2 included. The dependent variable I was interested in was the incubation duration, the number of days from first egg laid to first egg hatched. The results of the ANOVA were that Trial 1 hatchling success and Trial 2 temperature condition had significant effects; $F_{(1,27)}$, p = .0056, $F_{(1,27)}$, p = .03. Successful pairs in Trial 1 had shorter incubation durations in Trial 2 than birds that were unsuccessful in Trial 1 (see Figure 6). Birds that incubated at 30°C in Trial 2 had shorter incubation durations than birds that incubated at 14°C (See Figure 4). Ambient temperature condition 1 trended towards a significant main effect; $F_{(1,27)}$, p = .07. The interaction between Trial 1 and Trial 2 was not significant; $F_{(1,27)}$ p = .69. There should be some caution taken when interpreting these results, as some groups have a small sample size of less than five pairs.

Reproductive success per trial and condition

Trial Number	Condition	Number of pairs with no eggs	Number of pairs with only eggs	Number of pairs with hatchlings	Total number of pairs
Trial 1	30 °C	2	6	10	18 *
	14 °C	6	8	4	18 *
Trial 2	30 °C: 30 °C		1	8	9
	30 °C: 14 °C		2	6	8
	14 °C: 30 °C	2	1	5	8
	14 °C: 14 °C		4	3	7
Total numbers for Trial 2	30 °C	2	2	13	17 *
	14 °C	0	7	10	15 *

Table 1: Developmental stages that pairs arrived at by the end of each trial. * One pair from Trial 1 in the 30 °C and three pairs from Trial 1 in the 14 °C condition were excluded from analyses as one individual of the pair died, and therefore the pair could not continue into Trial 2.
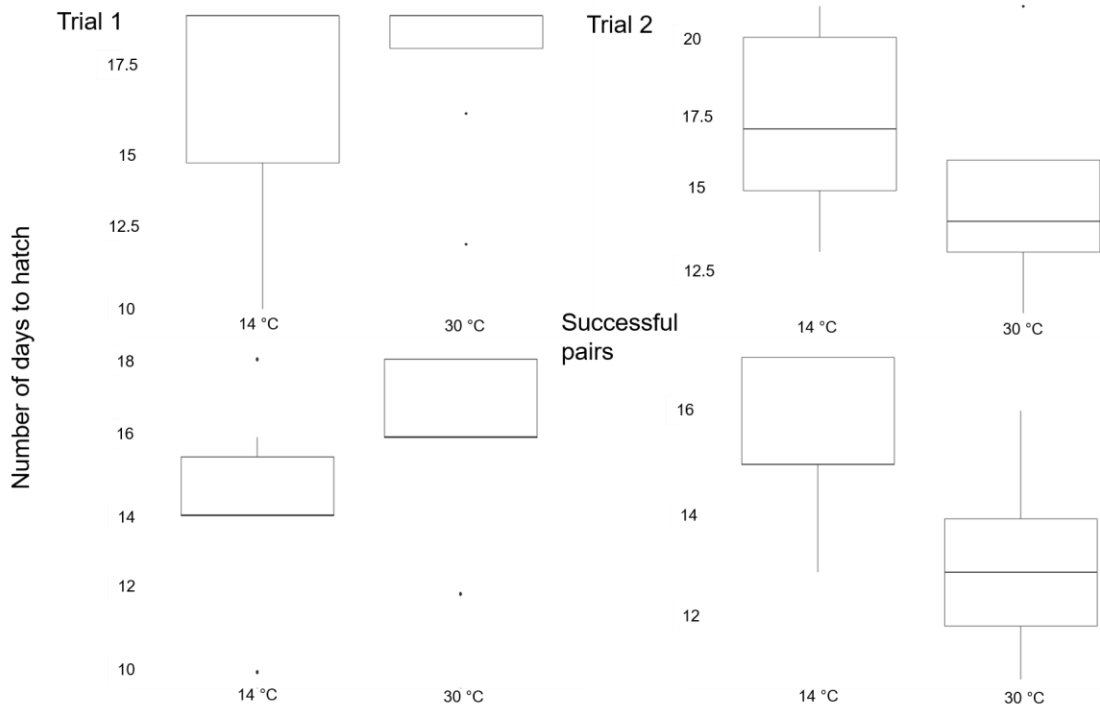
Figure 4. Incubation duration in days for each temperature condition. Trial 1 first column; Trial 2 second column. Bottom row is solely successful pairs. In Trial 1, pairs incubated significantly longer in the 30 °C condition as compared to the 14 ° C condition (z = -3.41, p = .0003). In Trial 2 they incubated longer in the 14 °C condition (z = -1.61, p = .05). The successful pairs show the same trend as the rest of the pairs.
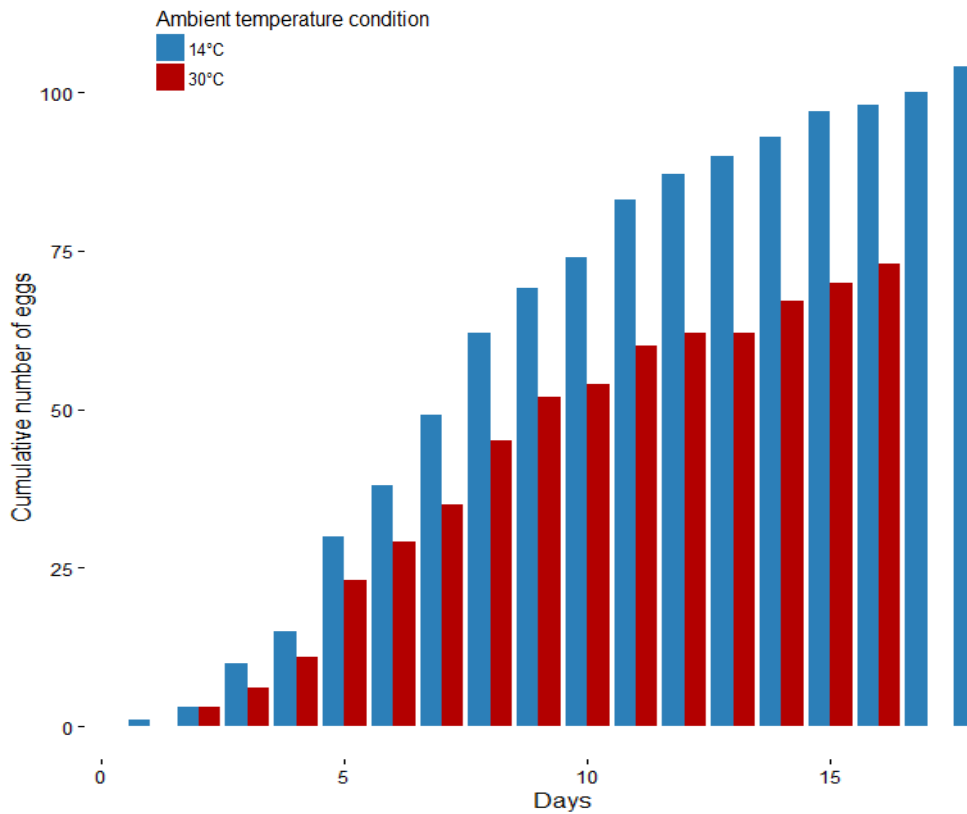
Figure 5. Cumulative number of eggs laid for Trial 2. Red shows eggs laid for the 30 °C condition, blue shows eggs laid in the 14 °C condition, both have a sample of 17 pairs per condition. The 14 °C condition laid earlier and more than the 30 °C condition. The 30 °C condition hatched prior to the 14 °C condition, which is why the blue bars continue further than the red bars. The hatch date was later in the 14 °C condition than the 30 °C.

To determine how Trial 1 hatchling success might have reduced Trial 2 incubation duration, I calculated mean nest temperature in Trial 2 at each time point throughout the day for each pair for all days of incubation for unsuccessful pairs and successful pairs for Trial 2. I did this separately for ambient temperature condition. The result was that birds successful in Trial 1, incubated at higher temperatures in Trial 2 than the unsuccessful pairs in Trial 1, irrespective of ambient temperature condition in Trial 2 as seen in an example of a representative incubation day (**Figure 7**).

As the nest temperature dataset was large, (~ 50,000 data points per ambient temperature condition), I used the whole distribution to see trends. The distributions of nest temperatures in Trial 2 for successful and unsuccessful birds from Trial 1 within the same temperature condition look quite different, with the unsuccessful birds having a peak in temperature around ambient temperature, whereas the successful birds have no such peak (**Figure 8**). The spread of nest temperatures is much higher in the 14 °C condition than the 30 °C (**Figure 9**). For nest temperature distributions for Trial 1 nest temperatures and separated by the success in Trial 1; and nest temperatures for Trial 2 and separated by the success in Trial 2 (as compared to **Figure 8** where the success in Trial 1 is compared to the nest temperatures in Trial 2), see **Appendix C**.
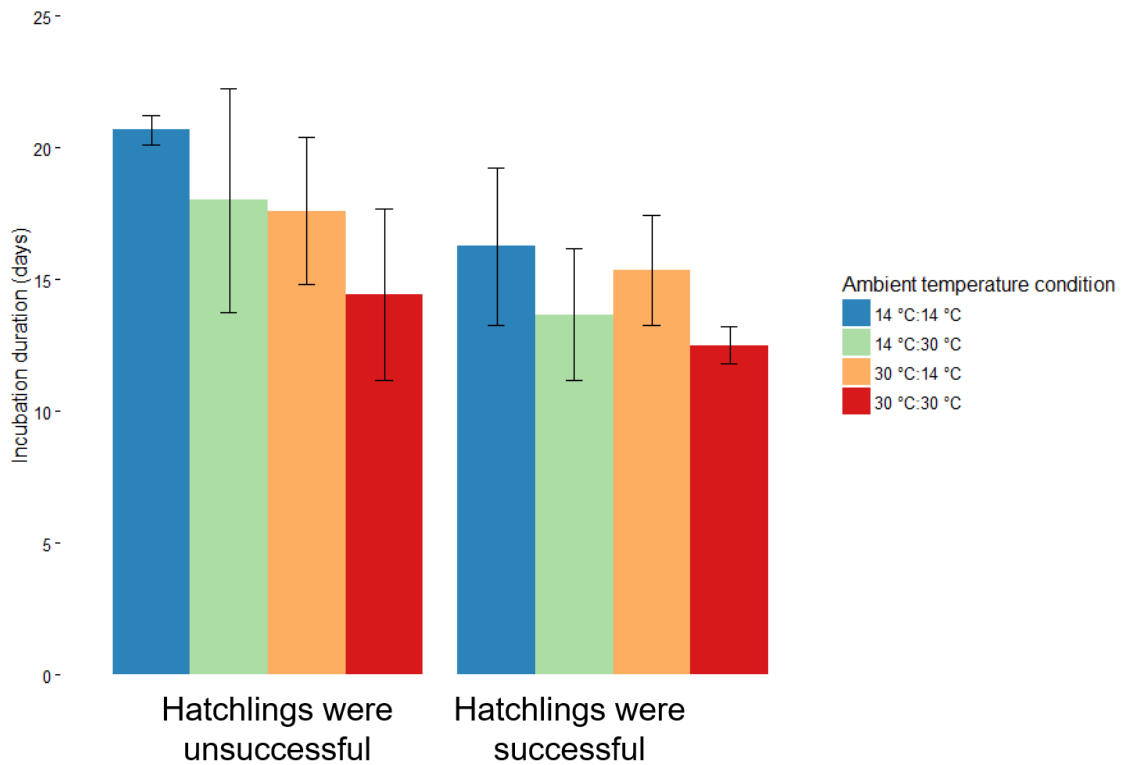
Figure 6. Mean values of incubation duration in Trial 2 for each of the ambient temperature groups, separated by previous hatchling success in Trial 1. The prediction was that pairs that had experience in the same ambient temperature condition, would need the same or less time to incubate and hatch offspring. Translating the prediction to the graph, I would expect the blue and red bars to be shorter than the green and orange bars, respectively. Although the 30 °C:30 °C and 30 °C:14 °C follow this pattern, the 14 °C:14 °C does not. The lack of significant effect in the ANOVA furthers the conclusion that at this sample size, there is likely not an experience effect. Hatchling success and ambient temperature condition in Trial 2 both had significant main effects on incubation duration for Trial 2. Error bars are standard deviation.
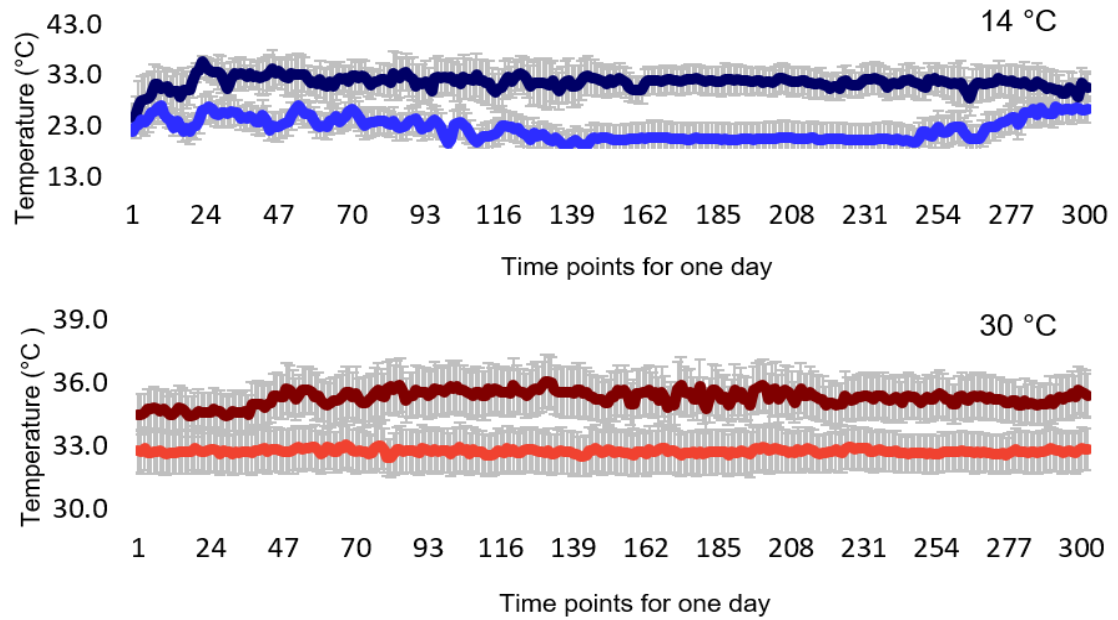
Figure 7. Mean incubation temperature as measured by the iButton for every pair and time point, for a representative incubation day. The temperatures for the whole incubation period is visualized in **Figure 8,9**. Pairs that were successful are shown in dark red and dark blue, with unsuccessful shown in light red and light blue (30 °C, and 14 °C, respectively). Successful pairs from Trial 1 maintain a higher temperature in the nest in Trial 2 than unsuccessful pairs. Error bars are standard error of the mean.

Nest temperature recordings for successful pairs in either ambient temperature condition

Nest temperature recordings for unsuccessful pairs in either ambient temperature condition
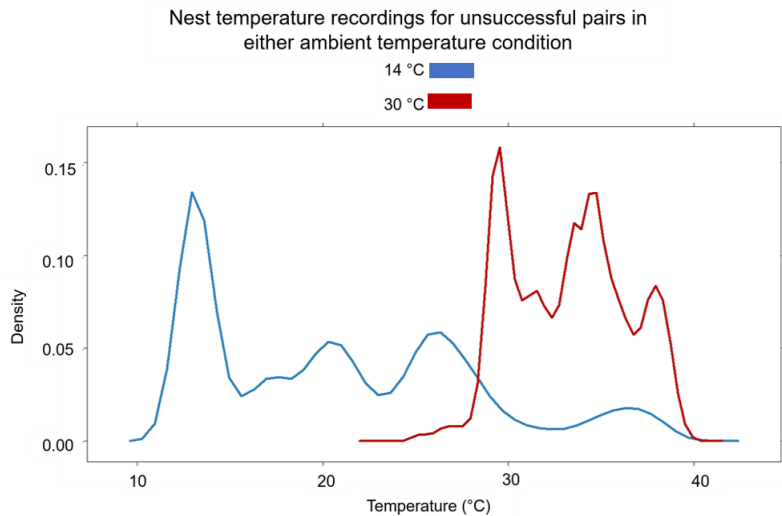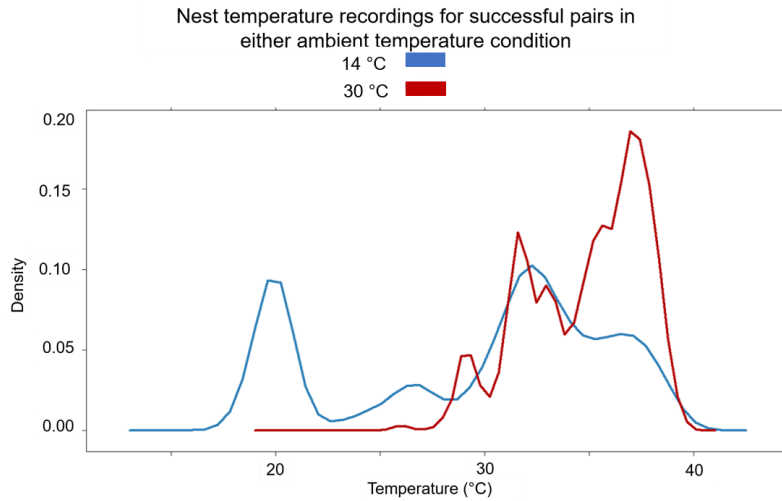
Figure 8. Nest temperature distributions for 30 °C and 14 °C for Trial 2, successful and unsuccessful from Trial 1 (top successful, bottom unsuccessful). Unsuccessful pairs have distributions with a large peak around ambient temperature. Density plots can be thought of as smoothed histograms. See **Appendix C** for successful and unsuccessful pairs from Trial 1 and the effect of success of Trial 2. Density plots have the number, in this case temperature value on the x-axis, and the y-axis has the probability density function of the kernel density estimation (Koehrsen, 2018). For more information, see https://towardsdatascience.com/histograms-and-density-plots-in-python-f6bda88f5ac0.
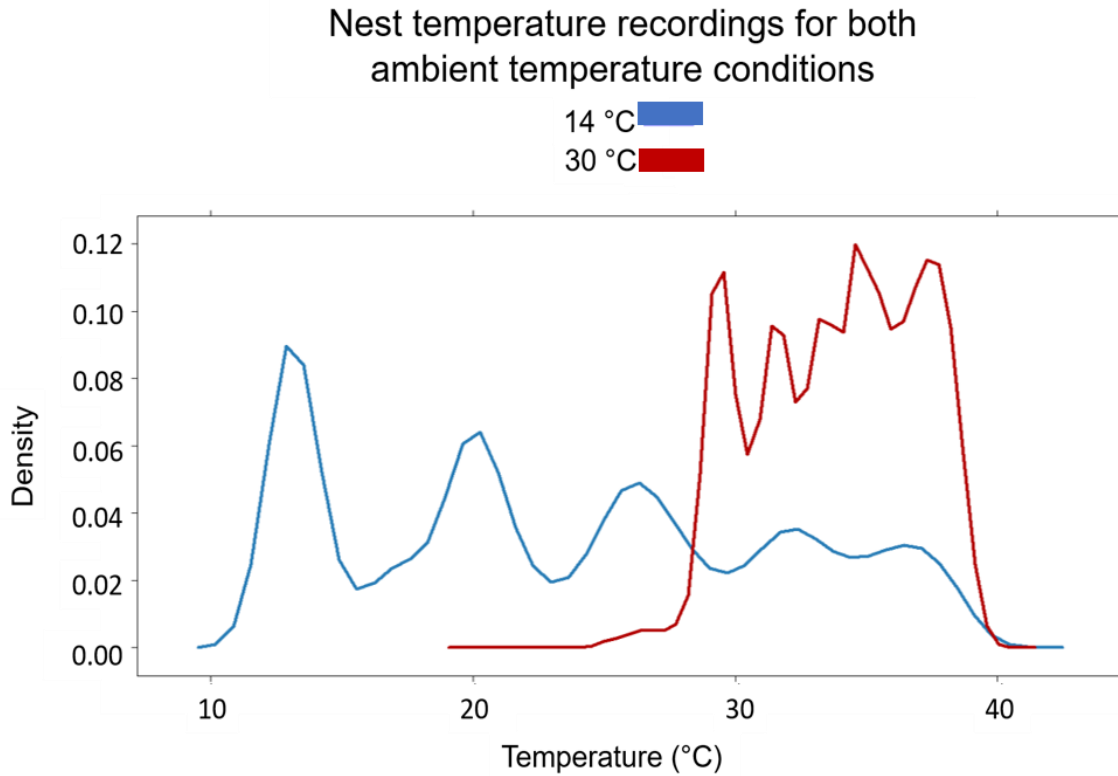
Figure 9. Distributions of temperature recordings for all pairs per condition, 30 °C in red, 14 °C in blue. Both distributions are significantly not normal (Anderson-Darling value of 573) and are visually quite different from each other, with the 30 °C nest temperature recordings having a narrow range of temperatures recorded, whereas the 14 °C is more dispersed. See **Appendix C** for a comparison of Trial 1 and Trial 2 complete temperature distributions.

3.2 HMM results

3.2.1 Descriptive results of the distribution

The normality of the distributions of the 30 °C and 14 °C nest temperature recordings for the total incubation period for all pairs were tested using the Anderson-Darling test statistic for normality. The critical value was 0.787, with the null hypothesis being that the data came from a normal distribution and the alternate hypothesis being the data does not come from a normal distribution. As the critical value was surpassed at 573, the null hypothesis was rejected, the data do not come from a normal distribution.

3.2.2 Training set creation

For the HMM to be successful, an accurate training set needs to be supplied to the HMM, to generate the transitional and starting probabilities that will lead to state predictions. I used three days of manually encoded behaviour for the training set. The larger the training set, the more information it has, however there is a tradeoff between manually encoding the data and the potential information gained. Therefore, three days of manual encoding was completed for three pairs in each condition (30 °C and 14 °C) for Trial 2. The pairs were tested using an ARMA regression to see if there were any individual differences in behavior, and as there were no significant differences between pairs in terms of behavior, the three pairs in each condition were pooled together to create two training sets: one for 14°C condition and the other for the 30°C condition (see **Figure 3** for behaviour state breakdowns between pairs). The purpose of the three pairs in the training set is to represent the population of temperature values for each behaviour state: one or two birds on the nest or neither. If one pair had a higher representation of one of the behaviour states, that still holds true; it is only if pairs have different temperatures for the same behaviour state where discrepancies from the pair to the population lie.

When manually encoding the behaviours, I synchronized the time recorded by the iButton to the time recorded by the video camera, so that the five-minute temperature sample the iButton recorded aligned with the behavior seen on the video. Initially, the behaviours were recorded with reference to the sex of the bird on the nest, however this proved not possible to predict using the model, so the training set was simplified to only

the number of birds on the nest. The three pairs were chosen with the best video resolution. I was blind to whether the pairs were successful previously in Trial 1 when encoding, and only recorded number of birds on the nest. The video resolution was insufficient to see whether the birds were sitting on the eggs or simply on the nest.

3.2.3 Temperature thresholds

In the initial implementation of the HMM, I used the nest temperatures, as recorded by the iButtons for both the training set and the test sequence. As nest temperature is a continuous variable (though measured discretely by the iButton to the nearest 0.5 °C), there was an insufficient number of recordings for each temperature point for the HMM to make adequate probabilities. As well, the probability distribution for all the unique temperature values did not appear to be the best way to encapsulate the data.

For these reasons, I used the mean to binarize the temperatures: 0 indicating at or below the mean, 1 indicating above the mean temperature of the training set. This gave the model more information to generate probabilities. However, there is a reduced resolution as the training temperatures are simplified to the point where fine resolution of temperature change is not possible, as seen in **Figure 10**. The quartiles and median were tested as potential thresholds, with no increase in sensitivity of the model to behavioural changes. Although the distributions were not normal, the mean was kept as a threshold as no better threshold was found.

3.2.4 HMM results

To test the accuracy of the HMM for predicting behavior from temperature recordings, I manually encoded one hour of incubation behavior for every possible pair in both ambient temperature conditions (seven pairs in 14 °C condition, nine pairs in 30 °C condition). The temperatures for the one hour segments were concatenated into two validation sets (one for each ambient temperature condition) of behavior and matching nest temperatures. The nest temperatures were fed into the HMM, and the predicted behaviours were compared to the manually recorded behaviours to assess how accurately the HMM could predict behaviour compared to manual encoding. The HMM predicted

one bird on the nest 100 % of the time for the 30 °C condition, and 98 % of the time for the 14 °C condition (see **Figure 11**). Comparing the predicted behaviours to the manually encoded behaviours led to 58 % and 66 % accuracy for the 30 °C and 14 °C conditions, respectively.

3.2.5 Allen's hummingbird methods and data

There is the possibility that the size of the training set is insufficient to create probabilities adequate to predict behaviours. I therefore used a data set of hummingbird incubation, courtesy of Erich Eberts, to test this possibility. The hummingbird training set was approximately 1000 data points collected from a thermal-imaging camera at a sampling rate of approximately 1 minute, a magnitude higher than the zebra finch training set as well as increased accuracy and precision. The hummingbird training set was also only of one female hummingbird, posing a simpler system of either 0 or 1 bird on the nest, as well as less individual differences as only one individual was analyzed. I used the same methodology to test the HMM behaviour predictions for the hummingbird data set as I did with the zebra finch data set, with a training set that was exclusive of a validation set, and where the validation set had known behaviours that the HMM was not privy to. The results shown in **Figure 14** illustrate that although the HMM was more accurate (92 %), the HMM still was unable to switch behavioural states akin to what was seen in the manual encoding of behaviour.
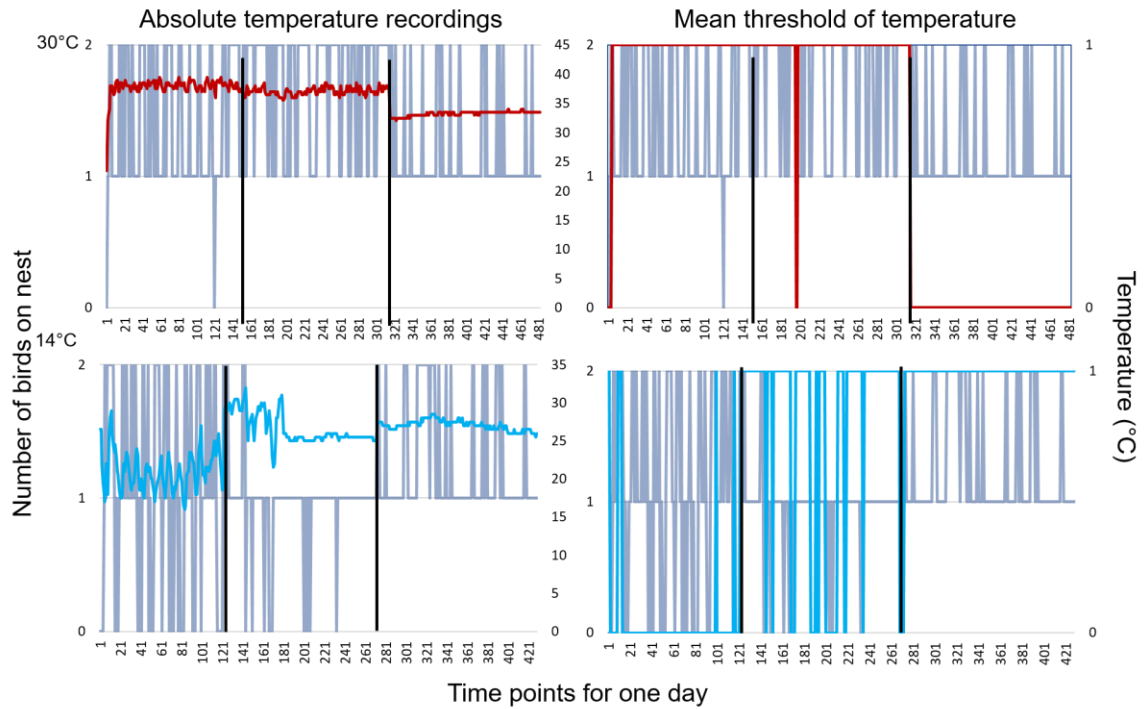
Figure 10. Recorded temperature for the training data set compared to binarized temperature shown below. Recorded temperatures are shown in the left panels with the temperature scale on the right side of each panel. Binarized temperatures are shown in the right panels with a binarized temperature scale. 30 ℃ training set shown in dark red (above), 14 °C training set shown in cyan (below). Grey lines indicate behaviour states, with number of birds on the left side of each panel. Black lines separate data for the three training data set pairs. For each pair, the record for one day randomly picked during the incubation period is shown. The binarized temperatures create a simpler model, but without the resolution of the absolute temperatures.
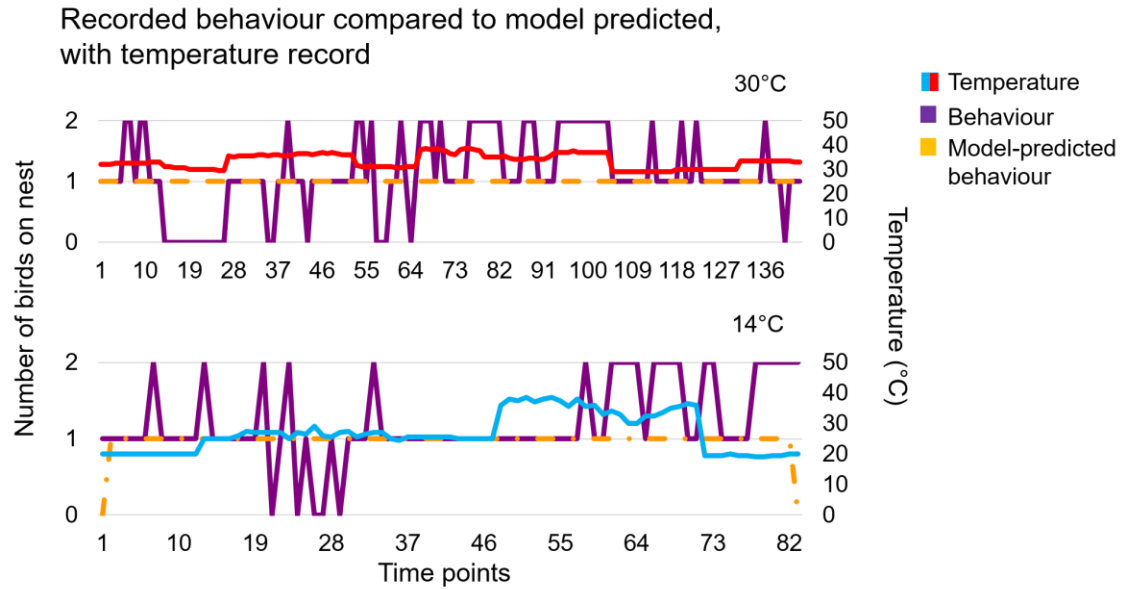
Figure 11. HMM predicted behavior (dashed orange) as compared to manually encoded behaviours (purple) and associated temperatures shown in red and cyan for the separate ambient temperature conditions. The HMM was unable to effectively capture the variation of behaviours.
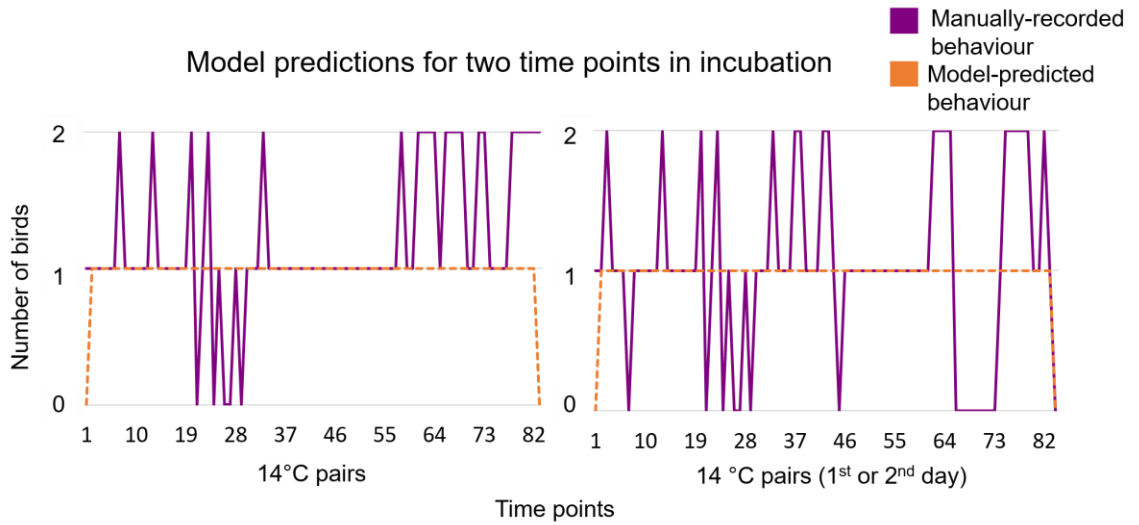
Figure 12. Comparing iButton recordings at random points during incubation (left) and at start of incubation (right). Model predictions remained constant, even as behavior changed. There was a decrease in accuracy of predicting behavior from temperature during the first few days. This was likely to more variable behavior that the model could not account for.
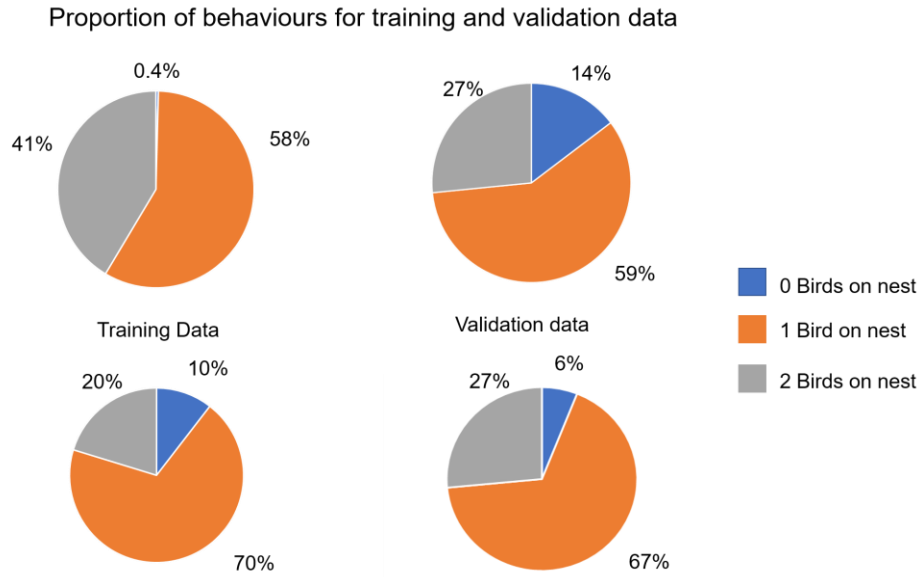
Figure 13. Proportion of behaviour for 30 °C (top two pie charts) and 14 °C conditions for the training set (left) and validation set (right). The training set is quite different in the 30 °C condition, however is similar in the 14 °C training set, with no associated increase in accuracy.
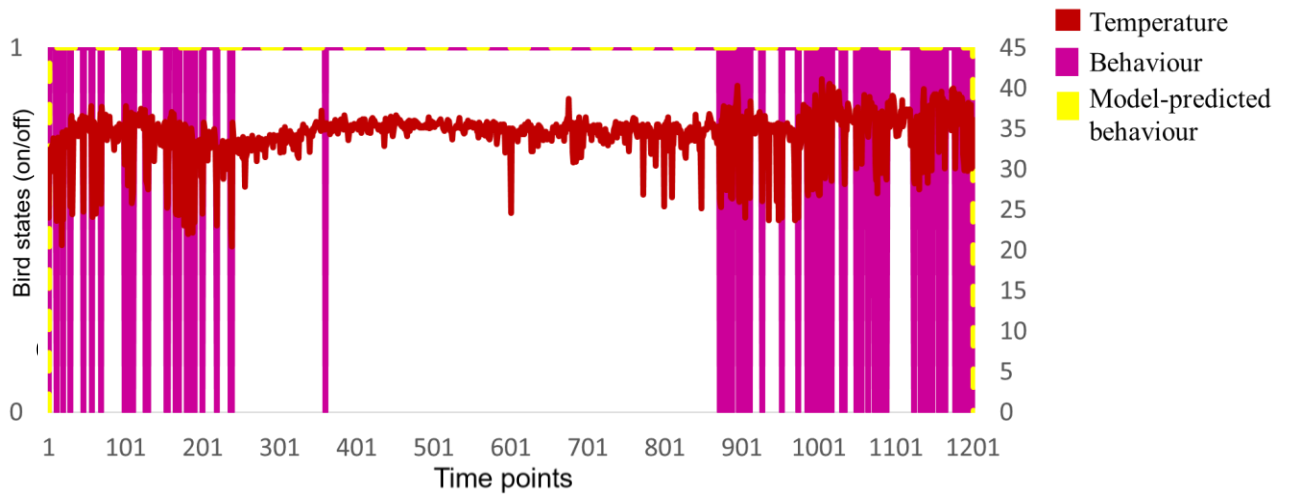
Figure 14. Hummingbird incubation, model predicted behaviour (dashed orange) still predicting one bird on the nest almost all the time, whereas the manually encoded behaviour (purple) shows instances of the hummingbird off the nest, with respective temperature variation (red). Even with an increased sample size, the HMM fails to encapsulate minute changes in behaviour.
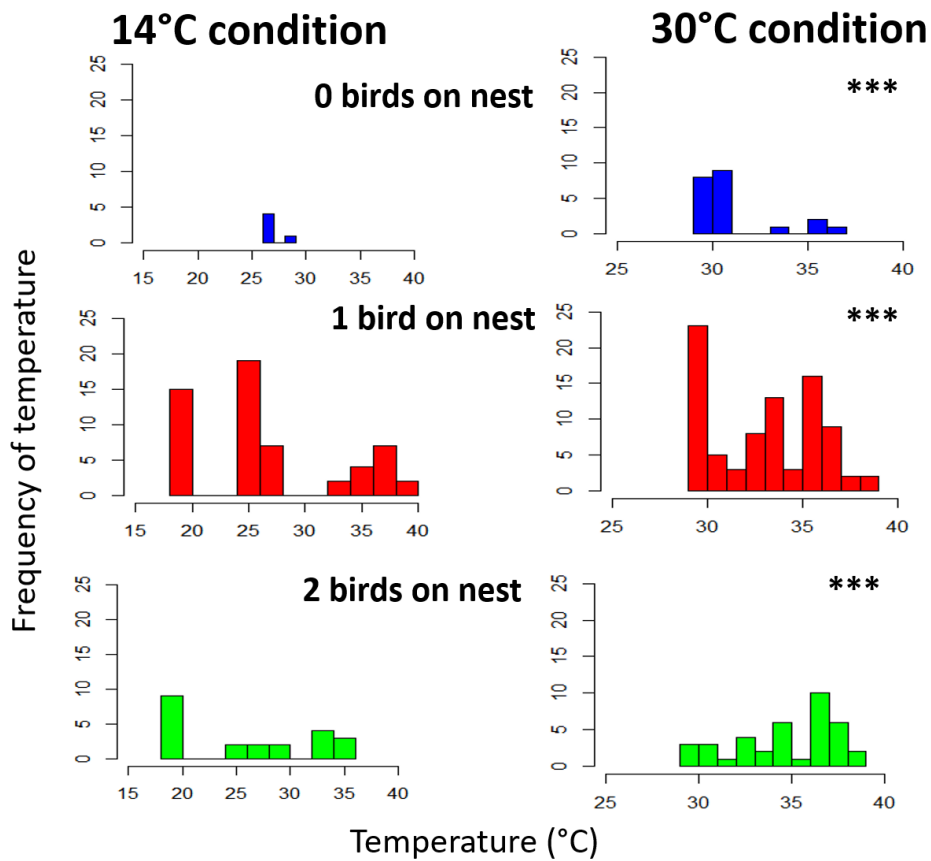
Figure 15. Distributions of whole temperature data set for each behaviour state, for each ambient temperature condition. There is an insufficient amount of data for zero birds on the nest to draw conclusions, and there is considerable overlap between 1 and 2 birds on the nest, which would make accurate prediction of behaviour using temperature not possible. Mann-Whitney tests with Bonferroni correction for multiple comparisons were done to see if there was a relationship between the behaviour (0,1, or 2 birds on nest) with the nest temperature distributions (14 °C 0 birds: 1 bird $z = -0.78$, $p = 1$, 1:2 $z = -0.398$, $p = 1$, 0:2 $z = 0.763$, $p = 1$; 30 °C 0:1 $z = -1$, *** $p < .001$, 1:2 $z = -4.19$, *** $p < .001$, 0:2 $z = -3.09$, *** $p < .001$).

Chapter 4: Discussion

4.1 Specific interpretations of results

4.1.1 Interpretation of breeding results

I hypothesized that birds would adapt incubation behaviour to ambient temperature conditions and their previous temperature-related experience. One prediction was that as 30 °C was close to 35 °C—the ideal temperature to incubate—that the incubation duration would be shorter for the 30 °C condition as compared to the 14 °C condition, this prediction has been supported by previous literature showing the lower the temperature, the longer the incubation duration (Carter, Hopkins, Moore, & Durant, 2014). The results were counterintuitive, the first trial birds in the 30 °C condition took more days to incubate than the 14 °C condition. Then the trend reversed for the second trial, with the 30 °C condition taking less days than the 14 °C condition. Even when selecting only pairs that were successful, the trend remained in both trials. Both conditions had the same breeding and temperature experience, and for the second trial breeding success was counterbalanced as to not be disproportionately affecting the interpretation of one ambient temperature condition as shorter than the other solely due to previous breeding success. The main effects on incubation duration were previous success and current ambient temperature condition, even when an interaction was expected. The condition the pair was in for Trial 1 had no significant effect on incubation duration for Trial 2, however the effect of previous temperature-related experience could still be present, but yet the effect may not have been strong enough to be significant. Replication and a larger sample size may yield significant results.

A possible interpretation of this result is that incubation duration is an indirect measure of nest attentiveness, the shorter number of days required to incubate, the more the parents sat on the nest, which is related to attentiveness (Weathers & Sullivan, 1989). As 30 °C is close to 35 °C the cost of being inattentive to the nest, cost being chance the eggs will die, is low and therefore to conserve energy the birds did not sit on the nest to the extent expected. In the 14 °C condition, leaving the nest had a higher chance of

cooling the eggs to the point of failure, therefore sitting on the nest is much more crucial. Although, this is not what was reported for Trial 1, with the 30 °C condition taking more days to hatch an egg than the 14 °C condition. For Trial 2, all pairs had more breeding experience than Trial 1 and may have responded to the lower cost of sitting too much on the nest compared to the cost of sitting too infrequently.

Successful pairs could maintain temperatures consistently higher than unsuccessful pairs, irrespective of ambient temperature condition and temperature-related experience. This result can be attributed to a combination of items, whether it was the type of nest the successful birds built was more insulative, or the successful birds were able to translate more of the skills learnt in Trial 1 to Trial 2. This can best be seen in the density plots of the distributions of ambient temperatures recorded by the successful and unsuccessful pairs (**Figure 8**), as the successful pairs' nests are rarely recorded at temperatures near the ambient temperature, whereas unsuccessful pairs have a large peak at ambient. Two variables that also affect the success of the birds have been shown to be quality of the female, as demonstrated with the number of eggs she lays, and the clutch size (Hanssen, Erikstad, Johnsen, & Bustnes, 2003). Successful pairs may have had females that harmonized the number of eggs laid with the feasible number of eggs the parents could take care of, in terms of energy expenditure.

4.1.2 Exploring why the HMM was unsuccessful at predicting behavior accurately

There are many reasons the HMM may have been inaccurate. When artificial data sets that I constructed with known probability distributions were fed into the HMM, the HMM performed as expected. When only one state was used in an artificial data set, the HMM was 100% accurate. When alternating between two states with a known probability of 50% (akin to flipping a coin), the HMM predicted the alternating behaviour states as expected. However, when using an artificial data set that had three behavioural states and only two observations paired with the three states (as was the case for the binarized temperature data), the third state was never predicted. This led to testing a 2-state HMM of only 1 bird on the nest or 0 birds on the nest, the same result of 1 bird on the nest 100% of the time was still found.

The inaccuracy of the HMM model could be due to a software error. Two different HMM programs, one from GitHub (https://github.com/jason2506/PythonHMM) and the other using the seqlearn package, set up by Dr. M. Daley (attached to Appendix A), were created using different functions and packages to see if the behavior predictions were due to the method in which different functions calculate probabilities. Both, however, produced identical results. It was unlikely that a software error was what was leading to the inaccurate predictions.

The iButtons could have recorded temperatures that were more constant than actual nest temperature. As the iButton was placed once the first egg was laid and seldom moved, the iButton could have slowly been enveloped by the nest or buried by the birds. The farther the iButton is from the clutch of eggs, the less accurate the temperature recording would be. To test this scenario, I manually encoded a subsequent validation set of either the first or second day of incubation. The first days of incubation would be the highest accuracy of the iButton as it was just recently placed in the nest. These first few days are also rife with activity as the birds have not laid their whole clutch and are not incubating as consistently as seen further along in incubation. If the inaccuracy of the iButton reading was the cause of the inaccuracy of the HMM predictions, there would be an increase in accuracy during these first few days. Instead, there was a minor decrease in accuracy from 66 % to 58 %, as seen in **Figure 12**. iButton inaccuracy may have added a small amount of error to the temperature readings as they were not guaranteed to be right beside the eggs, however they likely were not a large contribution to why the HMM was not successful at predicting behavior.

As the transition and the starting distribution relied on the training set to be an accurate representation of the data, it is possible that if the training set was not generalizable to the full data set, it would have led to erroneous behavior predictions. I first compared the proportions of behaviours seen in the training set and the validation sets and saw there was an undersampling of certain behaviours in the 30 °C condition (**Figure 13**). However, the training set and validation set for the 14 °C condition were similar, and yet the HMM was no more successful at predicting behaviours for the 14 °C validation set.

As the HMM assumes that in the training set each transition from state and temperature to the next state and temperature is the same interval apart and from the same individual, even though the pairs are more or less the same there is the transition from one pair to the next pair that is an erroneous transition. The method to fix the erroneous transition would be to treat each pair separately, however the training set is not large enough to accomplish accurate probabilities without concatenating the pairs. There are only two transitions from one pair to the next, and are unlikely to be causing the unsuccessful prediction of behaviour.

As detailed in the Temperature threshold section (3.2.3), there may be a more appropriate threshold to use as the binary above and below mean loses the detail of the absolute temperature-- potentially to the detriment of predicting behaviours. Quartiles and medians were also used, but with no increase in prediction accuracy.

My conclusion is that the assumption of a near fidelity between temperature and behaviour is false for zebra finch incubation, although other research has shown an extensive relationship between nest temperature and incubation behaviour (Coe, Beck, Chin, Jachowski, & Hopkins, 2015). I assumed that when the bird left the nest, shortly after the temperature would drop; when the bird comes back, the temperature would begin to rise. If my assumption were true, then there would be distinct distributions of temperatures that aligned with 0,1, and 2 birds being on the nest. However, if the birds are able to maintain a near constant temperature, while changing their behaviour, the HMM would not be able to handle this discrepancy. As with the successfully applied HMM detailed in section 1.6, each had a distinct behaviour and associated separate response variable. I tested to see if the mutually exclusive behaviour states and respective temperatures were present in the zebra finch data set. The nest temperature was not mutually exclusive to the behaviour state (see **Figure 15**). Although the 30 °C condition did show significant exclusivity between behaviour states, the range of the distribution is still too overlapping between the behaviour states for accurate predictions using temperature to be possible, as tested by Mann-Whitney tests with Bonferroni correction for multiple comparisons (14 °C 0 birds: 1 bird $z = -0.78$, $p = 1$, 1:2 $z = -0.398$, $p = 1$, 0:2

z = 0.763, p = 1; 30 °C 0:1 z = -1, p < .001, 1:2 z = -4.19, p < .001, 0:2 z = -3.09, p < .001).

4.1.3 Ramifications of HMM results

The HMM predicted one bird on the nest constantly. This is most likely due to the distribution of temperatures within the training set so that the HMM generated transition probabilities that once one bird was on the nest the probability to stay in the state was so high and to change to any other state was so low that one bird on the nest became a loop of predicted behaviour states. In general, birds rarely get off the nest, and when they do it seems to be with an insufficient amount of time to warrant a temperature change sufficient to have a discrete behaviour, as shown in **Figure 15**. This relationship between state and observation is integral for the HMM to be successful, as the examples where HMMs have been successful have discrete and mutually exclusive behaviours that have corresponding observations (see section 1.6). The insulation properties of the nest may have meant that the time birds spent off the nest had little effect on nest temperature which would explain why the temperatures did not correspond directly to behaviours.

A HMM was chosen as it is a relatively simple model, and if a simple model can encapsulate the phenomenon of interest, then there is no need to use a complex model. However, I have shown here that a HMM is insufficient to encapsulate the minute, and subtle behaviours that occur with little to no temperature change. As the quote generally paraphrased from George Box (1976) says: All models are wrong, but some are useful. Useful models to try in the future would use the full known incubation temperature and update the probabilities at each time point. As well using known factors like nest shape, and clutch size would better inform the model for more accurate predictions.

4.2 Future directions

There are branches that these results can be expanded upon. Similar to the hummingbird data set, replicating zebra finch incubation with a more precise instrument such as a thermal-imaging camera or thermocouple (nestled in a dummy egg) that records temperature continuously would allow for visualization of the heat gradient coming off

the nest. A thermocouple would also help test whether the birds are changing their heat output during incubation (see section 1.2)

Zebra finches were chosen as they are opportunistic breeders, and would have been most likely to exhibit behavioural changes. Now that we know these changes are possible over a short period of time, replicating this study with a species that is from a region with more stable temperatures, and thus less likely to exhibit behavioural changes with such ease would be worthwhile. This would help address whether these behavioural changes are a function of flexibility that zebra finches and other species such as the Adelie penguin and common eider have been selected for over time in more extreme climates, or are representative of conserved flexibility found homogenously throughout a broad range of birds.

The current study is a lab study and was able to show that only one cue, temperature, was sufficient to elicit behavioural change. It would be interesting to go back to field studies and do analyses to see what is the necessary cues for behavioural change. As well, these birds have been raised in captivity, therefore their ability to create nests and reproduce may be different than the wildtype. Comparing the reproduction of the captive compared to the wildtype would aid in understanding what traits have been conserved in regard to nest-building and incubation.

4.3 General conclusions

Many avian species are able to modulate incubation behaviour to be successful at the range of temperatures they naturally experienced. Adelie penguins, zebra finches and others have been shown to adjust the number of days required for incubation in concert with the temperature. The length of time required for the 30 °C condition in Trial 1 is puzzling, as I expected that the 30 °C condition, being so close to the ideal of approximately 35 °C would require fewer days to hatch and egg than the 14 °C condition.

There is a stark difference between the successful pairs and the unsuccessful pairs in their ability to maintain a higher temperature irrespective of ambient temperature condition. The combination of nest shape, breeding experience, incubation behaviour and

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body morphology likely gives rise to this difference. In all, what can be taken at face value of a hard physiological limit—the amount of time required to hatch a chick – is much more malleable to the parents' behaviour than once thought.

The HMM, although shown here not to be the appropriate model for this data set, has yielded an interesting and nuanced conclusion about avian incubation. Birds have a mechanism to maintain constant nest temperature, while not necessarily sitting on the nest, whether that is the duration of incubation periods or how the nest was built. The time spent away the nest is a function of the heat output by the bird and the heat retained by the nest, nonetheless the birds must have a method to discern when to come back to the nest when predation is not a factor. In general, the use of models in animal behaviour allow for an extensive amount of data collection and in-depth analysis. There is a need for an understandable model that can take input from a variety of data collection devices and output understandable metrics. That way, fine scale animal behaviour data can be collected in a systematic and objective manner, which will yield to more robust findings.

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Appendices

Appendix A: Two HMM codes, one adapted from Github link, the other from the seqlearn package, as set up by Dr. M. Daley

Github code

```python
from hmm import Model
from hmm import train
import matplotlib.pyplot as plt
import numpy as np
import csv
from scipy.stats import anderson
# import data as arrays

# Training data
myData = np.array(list(csv.reader(open('MANOVA-edit5.csv', 'r'))))
Temp = myData[:, 2].astype(float)
Behaviour = myData[:, 3].astype(str)

'''
# statistics on distribution
num_bins = 40
a = anderson(all_temp, dist = 'norm')
print a

fig4 = plt.figure()
ax4 = fig4.add_subplot(1, 1, 1)
n, bins, patches = ax4.hist(all_temp, num_bins)
ax4.set_xlabel('All temp')
ax4.set_ylabel('Frequency')
'''
# potential thresholding values
all_temp_mean = np.mean(all_temp)
all_temp_median = np.median(all_temp)
all_temp_1st = np.percentile(all_temp, 25)
all_temp_3rd = np.percentile(all_temp, 75)

# Test data
myData = np.array(list(csv.reader(open('iH17.csv', 'r'))))
Temp_Test = myData[:3570, 2].astype(float)
#test_hist = plt.hist(Temp_Test)

# Sort data into different pairs
# states
hot_behaviour_1 = Behaviour[0:147]
hot_behaviour_2 = Behaviour[147:315]
hot_behaviour_3 = Behaviour[315:483]
cold_behaviour_1 = Behaviour[483:605]
cold_behaviour_2 = Behaviour[605:755]
cold_behaviour_3 = Behaviour[755:912]
hot_behaviour = Behaviour[0:483]
cold_behaviour = Behaviour[483:912]
# symbols
hot = []
hot_1 = []
hot_2 = []
hot_3 = []
```

```python
test_sequence_h = []
hot_temp_1 = Temp[0:147]
hot_temp_2 = Temp[147:315]
hot_temp_3 = Temp[315:483]
'''
# binarize, can probably nest these guys all together
for i in hot_temp_1:
    if i <= all_temp_1st:
        hot_1.append(0)
    else:
        hot_1.append(1)

for i in hot_temp_2:
    if i <= all_temp_1st:
        hot_2.append(0)
    else:
        hot_2.append(1)

for i in hot_temp_3:
    if i <= all_temp_1st:
        hot_3.append(0)
    else:
        hot_3.append(1)
'''
# binarize test sequence
for i in Temp_Test:
    if i <= all_temp_median:
        test_sequence_h.append(0)
    else:
        test_sequence_h.append(1)

# binarize training without pair distinction
for i in Temp:
    if i < all_temp_median:
        hot.append(0)
    else:
        hot.append(1)

sequence_h = [hot_behaviour,hot]
print sequence_h

sequence_c = [
    (cold_behaviour_1, cold_temp_1),
    (cold_behaviour_2, cold_temp_2),
    (cold_behaviour_3, cold_temp_3)
]

# train model on pairs data
model_h = train(sequence_h, smoothing =1)

#model_c = train(sequence_c)

#test_sequence_h =

#[38,37,36.5,36.5,36.5,37,37,37,38,38,37.5,37.5,37.5,37.5,37.5,37.5,37.5,38,38,
38.5,38.5,38.5,38.5,38,
     #
38,38,38,23.5,38,38,38,23.5,23.5,23.5,23.5,37.5,37.5,37.5,37.5,37.5,37.5,37.5,3
7.5,37,37,36.5,37.5,37.5,37]
#test_sequence_c = [0,1,1,1,0,0,0,0,1,1,1,1,1,1,1,1,1]

#Create probability matrix
prediction_h = model_h.decode(test_sequence_h)
```

```python
print model_h
print model_h.evaluate(test_sequence_h)
print prediction_h
print len(sequence_h)

time_H3 = range(0,len(test_sequence_h))
'''
plot = plt.plot(time_H3, test_sequence_h)

#print prediction_h
for time_H3, prediction_h in enumerate(prediction_h): #code fixed by Philip
Todd Coppola.
    if prediction_h == 'B':
        plt.vlines([time_H3], 10, 50, alpha=.7)
#plt.show()
'''
```

seqlearn code

```python
# -*- coding: utf-8 -*-
"""
Created on Thu Oct 18 10:07:58 2018

@author: Daley
"""
import numpy as np
import seqlearn.hmm
import csv
import numpy as np

# import data
myData = np.array(list(csv.reader(open('cold.csv', 'r'))))
testData = np.array(list(csv.reader(open('validation_cold.csv', 'r'))))
# all cold temperatures extracted
cold_Temp = myData[:, 2].astype(float)
# test temperatures
testTemp = testData[:, 1].astype(float) #don't have any test temperatures yet

# all behaviours
cold_behaviour = myData[:, 3].astype(str)
test_Behaviour = testData[:, 2].astype(str)

# initialize what will become categorical numerical behaviours
num_beh = []

# change behaviours from char --> num
for i in cold_behaviour:
    if i == 'B':
        num_beh.append(1)
    elif i == 'M':
        num_beh.append(1)
    elif i == 'F':
        num_beh.append(1)
    else:
        num_beh.append(0)

# change continuous temp --> binary
binary_h = []
binary_c = []
temp_mean = np.mean(cold_Temp) # using mean for now, can change to different
threshold
temp_1st = np.percentile(cold_Temp, 25)
```

```python
temp_3rd = np.percentile(cold_Temp, 75)
temp_med = np.median(cold_Temp)
for i in cold_Temp:
    if i <= temp_mean:
        binary_h.append(0)
    else:
        binary_h.append(1)

# create inverse hot
binary_c = [1 - x for x in binary_h]

'''
for i in binary_h:
    if i == 0:
        binary_c.append(3)
    elif i == 1:
        binary_c.append(2)
    elif i == 2:
        binary_c.append(1)
    else:
        binary_c.append(0)

'''
# change continuous temp --> binary
test_h = []
test_c = []

for i in testTemp:
    if i <= temp_mean:
        test_h.append(0)
    else:
        test_h.append(1)
'''
for i in testTemp:
    if i <= temp_1st:
        test_h.append(0)
    elif i <= temp_med:
        test_h.append(1)
    elif i <= temp_3rd:
        test_h.append(2)
    else:
        test_h.append(3)
'''
# create inverse hot
test_c = [1 - x for x in test_h]
'''
for i in test_h:
    if i == 0:
        test_c.append(3)
    elif i == 1:
        test_c.append(2)
    elif i == 2:
        test_c.append(1)
    else:
        test_c.append(0)
'''
#  put together in one matrix
bin_temp = (np.vstack([binary_h,binary_c])).T
bin_test = (np.vstack([test_h,test_c])).T
# set parameters for HMM
hmm = seqlearn.hmm.MultinomialHMM(decode='viterbi', alpha=0.01) # try these??
Should be good
```

```python
# fit with data, train??
hmm.fit(bin_temp,num_beh,bin_temp.shape[0])

# test sequence
test_birds = hmm.predict(bin_test)

print(test_birds)
np.savetxt("testbirds.csv", test_birds, delimiter=",")
'''
# Let's assume we have two temperatures: 'cold' and 'hot'
# We're going to use 'one hot' encoding which means that we will have three
# seperate arrays... one for each temperature level... and at each time point
# only *one* array will have '1' (because you can't be hot and cold at the same
time!)

# Like this (I'm just making this data up, of course)
hot =
np.array([1,1,1,1,0,0,0,0,0,0,0,0,0,1,1,1,1,1,1,0,0,0,0,0,1,1,1,1,1,0,0,0,0])

# and since we know that whenever it's not hot, it has to be cold, I can
generate the 'cold' array without more typing
cold = 1-hot

# Now we combine this into our input matrix of format (n_samples, n_features)
temp = (np.vstack([hot,cold])).T

# And, finally, since this is *supervised* learning, we need the observations
of the
# hidden states. Let's make the observation the number of birds on the nest.

num_birds_on_nest = np.array([2, 2, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 2,
2, 1, 1, 0, 0, 0, 0, 0, 1, 2, 2, 1, 0, 0, 0, 0, 0])

# (I'm totally making this data up, of course)

# Now let's initialize our HMM

hmm = seqlearn.hmm.MultinomialHMM(decode='viterbi', alpha=0.01)

# And train it!

hmm.fit(temp,num_birds_on_nest,temp.shape[0])

# and now lets ask for a prediction based on the training data (which is an
absolute sin... but I'm doing it here just to show this works)

num_birds=hmm.predict(temp)
'''
# TODO
# - Get your temp data into one-hot format
# - Plug your real data and observations into this code
# - sanity check results!
```

Appendix B: Animal use protocol for zebra finches

Western 🛡️

2015-019:3:

AUP Number: 2015-019
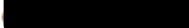**AUP Title:** Cognition, behavior, and the brain of birds.
Yearly Renewal Date: 10/01/2019

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2015-019 has been approved by the Animal Care Committee (ACC), and will be approved through to the above review date.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:

1) Animals used in this research project will be cared for in alignment with:
  a) Western's Senate MAPPs 7.12, 7.10, and 7.15
   http://www.uwo.ca/univsec/policies_procedures/research.html
  b) University Council on Animal Care Policies and related Animal Care Committee procedures
   http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.html
2) As per UCAC's Animal Use Protocols Policy,
  a) this AUP accurately represents intended animal use;
  b) external approvals associated with this AUP, including permits and scientific/departmental peer approvals, are complete and accurate;
  c) any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and
  d) AUP form submissions - Annual Protocol Renewals and Full AUP Renewals - will be submitted
   and attended to within timeframes outlined by the ACC. http://uwo.ca/research/services/animalethics/animal_use_protocols.html
3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will
  a) be made familiar with and have direct access to this AUP;
  b) complete all required CCAC mandatory training (training@uwo.ca); and
  c) be overseen by me to ensure appropriate care and use of animals.
4) As per MAPP 7.15,
  a) Practice will align with approved AUP elements;
  b) Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;
  c) UCAC policies and related ACC procedures will be followed, including but not limited to:
    i) Research Animal Procurement
    ii) Animal Care and Use Records
    iii) Sick Animal Response
    iv) Continuing Care Visits
5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially
  exposed to hazardous materials will have completed in advance the appropriate institutional OH&S training, facility-level training, and reviewed related (M)SDS
Sheets, http://www.uwo.ca/hr/learning/required/index.html

Submitted by: ██████████
on behalf of the Animal Care Committee
University Council on Animal Care

The University of Western Ontario
Animal Care Committee / University Council on Animal Care
London, Ontario Canada N6A 5C1
519-661-2111 x 88792 Fax 519-661-2028
auspc@uwo.ca½ http://www.uwo.ca/research/services/animalethics/index.html

Appendix C: Trial 1 and post-Trial 2 nest temperature distributions for all pairs and separated by hatchling success
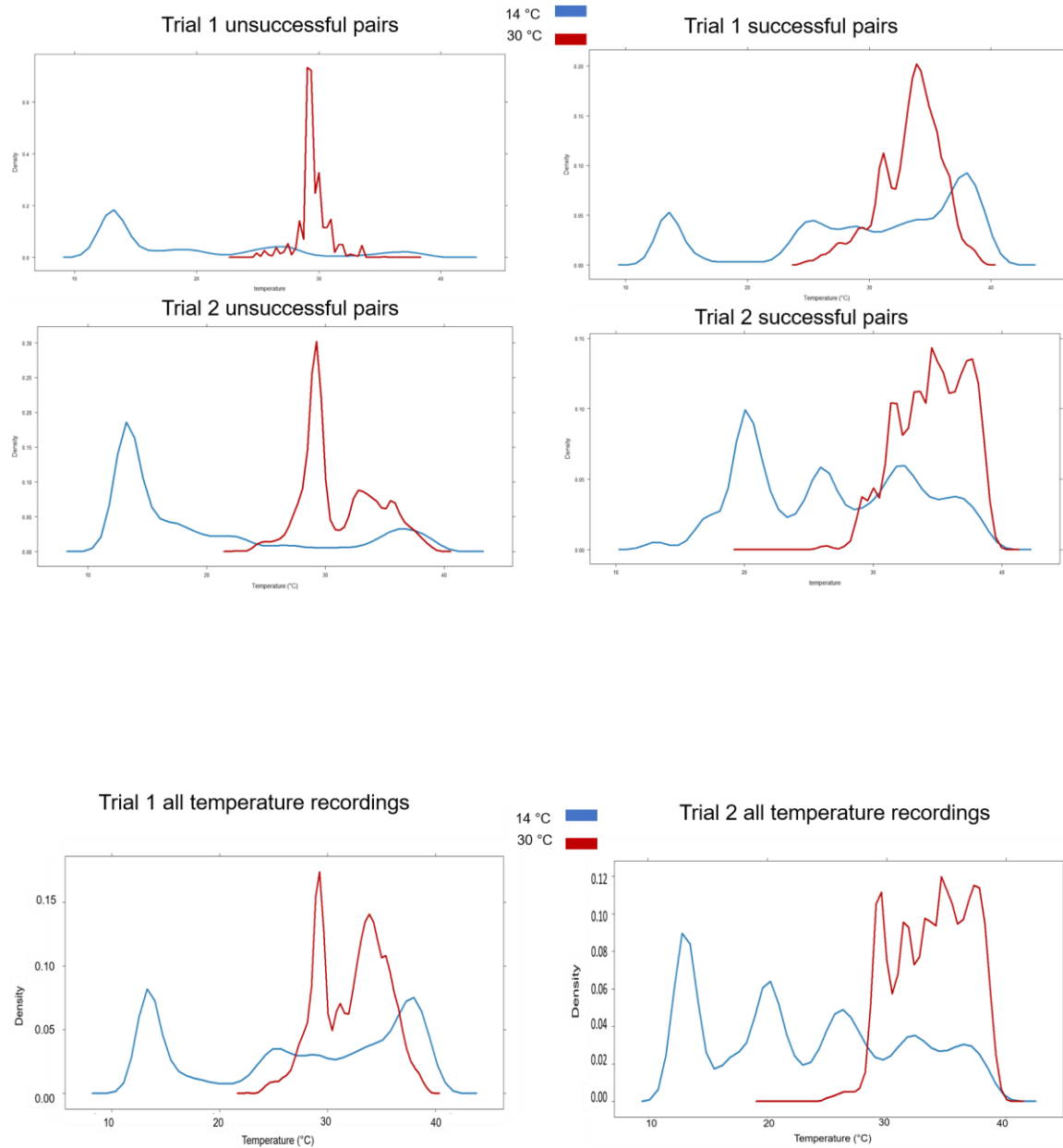
Curriculum Vitae

Tanya Shoot

EDUCATION

Master of Science: Neuroscience
2017-2019
The University of Western Ontario, London, Ontario

Bachelor of Science: Honours Specialization in Neuroscience
2013-2017
The University of Western Ontario, London, Ontario

Study Abroad: Neuroscience
2015-2016
The University of St. Andrews, St. Andrews, Fife, Scotland

RESEARCH EXPERIENCE

Thesis Student
Advanced Facility for Avian Research, London, Ontario
June 2016-April 2017
- Created a unique research question that will lead to at least one peer-reviewed, published scientific article
- Orchestrated the necessary specialists in the lab to aid in successful task completion
- Organized a timetable that lead to success in caring for 72 birds, school work and literature review
- Taught volunteer how to care for birds, observe their behaviour to identify injuries, provided constructive criticism and supervision as necessary, the volunteer can now care for birds on their own

Intern in Corneil Lab
The University of Western, Ontario, London
April-August 2015, July-August 2016
- Used Matlab software to create a program without any coding experience or training to analyze data effectively, which required independent learning through online courses and Matlab resources

Research Assistant in Healy Lab
The University of St. Andrews, St. Andrews, Scotland
January 2015-May 2016
- Cared for zebra finches and used light microscopy to analyze brains for activation, data will contribute to larger research into brain areas implicated in fine motor learning

Data Analyst
LUSO, London, Ontario
2014-2015
- Work in a predominantly Spanish-speaking environment with two years of Spanish classes, translate and create databases for client and statistical analysis of data, which then was published in the annual fiscal report

Research Methods Project
The University of Western Ontario, London, Ontario
2014-2015
- Created a unique research question to test perceptions of threats to a romantic relationship with the constraints of a small sample size of a mixed cohort that yielded a significant result when the paper was completed

CONFERENCES

Shoot, T.T., Martin, R. J., Sherry, D.F. (2017) "The role of temperature on nest structure in zebra finches" Western Student Research Conference (WSRC), London, Ontario, CA. [*Talk*]

Shoot, T.T., Martin, R. J., Sherry, D.F. (2017) "The role of temperature in zebra finch nest building" Comparative Cognition Society (CO3), Melbourne, Florida, USA. [*Poster*]

Shoot, T.T., Martin, R. J., Sherry, D.F. (2017) "Nest building under stress: How temperature affects nest structure in zebra finches" Animal Behaviour Society (ABS), Toronto, Ontario, CA. [*Poster*]

Shoot, T.T., Martin, R. J., Sherry, D.F. (2017) "Role of temperature in zebra finch nest structure and incubation" Biology Graduate Research Forum (BGRF), London, Ontario, CA. [*Talk*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2018) "Analyzing zebra finch incubation with computer algorithms" Western Research Forum (WRF), London, Ontario, CA. [*Poster*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2018) "Analyzing zebra finch incubation with a computer algorithm" Comparative Cognition Society (CO3), Melbourne, Florida, USA. [*Talk*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2018) "Can birds flexibly adapt incubation behaviour in varying environmental conditions?" Southern Ontario Neuroscience Association (SONA), Guelph, Ontario, CA. [*Poster*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2018) "Implication of temperature on incubation behaviour: A hidden Markov Model

method" Canadian Society of Ecology and Evolution (CSEE), Guelph, Ontario, CA. [*Poster*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2018) "Zebra finch incubation behaviour depends on ambient temperature and experience: A hidden Markov model approach" International Ornithological Conference (IOC), Vancouver, British Columbia, CA. [*Talk*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2019) "Understanding avian incubation with a computer algorithm" Conference on Comparative Cognition (CO3), Melbourne Beach, Florida, USA. [*Poster*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2019) "Avian incubation: how previous hatchling success and ambient temperature can affect behaviour" Ontario Ecology, Ethology, and Evolution Colloquium (OE3C), Hamilton, Ontario, CA. [*Talk*]

Shoot, T.T., Edwards, S.C., Martin, R. J., Healy, S.D., Sherry, D.F., & Daley, M.J. (2019) "Should I sit or should I go? Temperature and hatchling success affects avian incubation" Animal Behaviour Society (ABS), Chicago, Illinois, USA. [*Talk*]

HONOURS AND ACADEMIC AWARDS

Ontario Graduate Scholarship
2019-2020
The University of Western Ontario, academic accomplishment

Neuroscience Graduate Student Travel Award
2018, 2019
- *The University of Western Ontario,* academic accomplishment

Dean's Honour List
2013-present
- *The University of Western Ontario,* academic accomplishment

International Learning Award
2015
- *The University of Western Ontario*, for academic accomplishment and to financially aid study abroad term

Robert T. Jones Scholarship
2015
- *The University of Western Ontario,* for academic accomplishment and community involvement, study abroad in Scotland, continuation of degree

Western Scholarship of Distinction
2013
- *The University of Western Ontario,* academic accomplishment