August 2017

Winter Warming Affects the Onset of Reproduction but not Cognition or The Hippocampus in Black-capped Chickadees (Poecile atricapillus)

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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Abstract

The effects of overwinter temperature on behaviour and cognition, hippocampal volume, and reproductive timing were studied in Black-capped chickadees (*Poecile atricapillus*). I hypothesized that overwinter temperature would have varied effects for Black-capped chickadees that overwinter in northern climates. I found that temperature had no effect on behaviour and cognition, or hippocampal volume, but temperature did influence reproductive timing. Birds that experienced warmer winter conditions had significantly more developed gonads than birds that experienced colder conditions. These results suggest that while birds are clearly sensitive to ambient winter temperature, temperature only modulates some processes and not all. These studies provide the basis for continued exploration into the effects of environmental change on avian populations, and can help influence current and future conservation and policy decisions.
Keywords

Co-Authorship Statement

The work presented in this thesis was completed under the supervision of Dr. David F. Sherry. All three experiments presented were done in collaboration with Dr. David F. Sherry, who helped formulate and refine the project questions and goals. The research presented in this thesis was influenced by, and benefitted from, input and feedback from members of the Advanced Facility for Avian Research, and the Sherry Lab group. Specifically, input was used in refining the design of each experiment, as well as determining suitable statistical analyses for each project.
Acknowledgments

First and foremost, I would like to thank Dr. David F. Sherry for his leadership and supervision during the last two years. I would like to thank Dr. Sherry for providing an always welcoming academic environment that has allowed me to grow and develop as both a researcher and as a person. I look forward to continuing to work with Dr. Sherry in the future.

I would also like to thank my colleagues at the Advanced Facility for Avian Research (AFAR) and in the Biology Department. In particular, I would like to thank the members of the Sherry Lab group (past and present): Christopher Course, Caroline Strang, Madeleine Brodbeck, Gloria Cho, Hayden MacDonald, Jordan Phelps, Tanya Shoot, Emily Brown, Nicole Guitar and Sam Noyak, for your time, input, and expertise. I also express my gratitude to the many undergraduate research assistants that aided in the completion of my project. I would like to acknowledge Caitlin Sullivan, Melanie Boivin, and Vanessa Colton specifically. I would like to thank Dylan Baloun, Kevin Young and Dr. Ben Rubin for their assistance with the statistical analyses in my project and Andrea Boyer for assistance in editing and reviewing.

I would like to thank the AFAR Staff: Andrew Gould, Michela Rebuli, and Francis Boon, for the impact you had on my research. Specifically, I would like to thank Andrew Gould for the many hours spent troubleshooting logistical problems with me.

I would like to thank my colleagues for their unwavering support over the last two years. I was fortunate to have a great group of friends to share this experience with, and to those people I will be forever grateful. In particular, I would like to acknowledge Dylan Baloun and Jessica Deakin for their endless and unwavering support, both personally and professionally.
Perhaps most importantly, I thank my family. My parents, Scott and Rebecca Martin, for their support and understanding which afforded me the opportunity to pursue this degree. My grandparents, who took an interest in my work, and provided support whenever called upon. Lastly, my siblings, who have all at various times in the past two years, weighed in on my work or my life, and in some way kept me going.

Thank you all.
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<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer (micron)</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>6H</td>
<td>Treatment receiving 6 hours of additional heat</td>
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<tr>
<td>24H</td>
<td>Treatment receiving 24 hours of additional heat</td>
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<tr>
<td>AFAR</td>
<td>Advanced Facility for Avian Research</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>am</td>
<td>Ante meridiem; in the morning</td>
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<td>cm</td>
<td>Centimetre</td>
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<td>DCX</td>
<td>Doublecortin</td>
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<td>F</td>
<td>Female</td>
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<td>g</td>
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<td>GLM</td>
<td>General Linear Model</td>
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<td>M</td>
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<td>Milligram</td>
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<td>Minutes</td>
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<td>mm</td>
<td>Millimetre</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<tr>
<td>PBS/T</td>
<td>Phosphate Buffered Saline with Triton</td>
</tr>
<tr>
<td>pm</td>
<td>Post meridiem; in the afternoon</td>
</tr>
<tr>
<td>QMR</td>
<td>Quantitative Magnetic Resonance</td>
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<td>s</td>
<td>Seconds</td>
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1 General Introduction: Winter warming affects the onset of reproduction, but not cognition or the hippocampus, in Black-capped chickadees (*Poecile atricapillus*)

1.1 Black-capped chickadees

Black-capped chickadees (*Poecile atricapillus*) are small, non-migratory birds that inhabit a broad geographic range in North America (Foote et al., 2010; Figure 1.1). Chickadees feed on insects and other animal matter, as well as on fruits, berries, and seeds. The ratio of animal matter to plant matter in their diet changes throughout the year (Smith, 1991).

Although non-migrants, Black-capped chickadees follow a regular annual cycle with many distinct phases. These phases can be characterized by a series of life history events and distinct behaviours.

In the fall, chickadees begin to form winter flocks (Smith, 1991). Each flock can include between 2-12 chickadees, and consists mainly of male-female pairs (reviewed in Smith, 1991). Individuals in these flocks spend the fall establishing a linear dominance hierarchy. Also in the fall, chickadees begin to increase the use of a food caching strategy. Individuals can store, and remember the locations of, thousands of food items and are able to retrieve them at a later time (Sherry, 1984; Healy & Hurly, 2004). While chickadees have been observed caching many food types (Heinrich & Collins, 1983; Lawrence, 1958), the majority of caches contain seeds and other plant matter (Smith, 1991). This is likely due to the decline in available insect biomass in fall, and the greater longevity of plant matter such as seeds and nuts.

Throughout the winter season, Black-capped chickadees remain in their flocks, and the linear hierarchies formed in the fall have become stable (Smith, 1976). Individuals rely
heavily on cached food for winter survival because food that is abundant at other times of year is at its annual low. To combat cold winter temperatures, Black-capped chickadees employ multiple strategies. For example, Black-capped chickadee flocks will roost in a common location and huddle together for warmth (Smith, 1991). Black-capped chickadees will also use facultative hypothermia to combat cold winter temperatures (Chaplin, 1974; Grossman & West, 1977). Facultative hypothermia is a strategy by which individuals lower their core body temperature to reduce metabolic costs (as reviewed in McKechnie & Lovegrove, 2002).

Towards the end of winter and into spring, flocks start to break into breeding pairs (Smith, 1991) and once the flocks dissolve and territories are established, both males and females help in prospecting and excavating a nest cavity (Smith, 1991). Spring is also the time when reproduction occurs. Black-capped chickadee broods range from one to thirteen offspring, with broods between six and eight being the most common (Smith, 1991). In the event of an early brood, replacement and second broods have been documented in Black-capped chickadees (Smith, 1991). Throughout the spring, chickadees forage mostly on the newly emerging insects available, and significantly decrease their consumption of plant mass, transitioning to a more animal based diet (Smith, 1991). Although extra pair copulations do occur (Otter et al., 1994), Black-capped chickadees are socially monogamous and both males and females help raise young (Smith, 1991).

In the late spring and through the first part of summer, parents are dedicated to raising their young. Black-capped chickadees fledge at around 16 days post hatch, and all the chicks in a brood usually fledge on the same day (Smith, 1991). Once out of the nest, fledglings typically do not return to the nest (Smith, 1991), instead parents and offspring move together in a family unit throughout the breeding territory (Odum, 1941). Parents still provide for
their offspring post fledging, but this provisioning rate declines as the chicks become more self-sufficient. As the summer progresses, breeding territories become less defined, and family groups begin to move more freely across former territory boundaries (Smith, 1991).

In late summer and early fall, family groups begin to split as the juveniles disperse (Smith, 1991). It is at this point that the cycle begins to repeat itself, with the birds beginning to form their own wintering flocks, usually containing an adult pair and newly dispersed, unrelated juveniles.

1.2 Temperature Trends and Ecological Impacts

Most species, including many birds, are adapted to specific environments. As a result, environmentally specific adaptations can be found in various populations. While some of these adaptations are obvious (e.g. penguins lack flight but are able to swim), others are more subtle (e.g. Ground tits’ (*Parus humilis*) genetic adaptations to elevation; Qu et al., 2013). These environmental adaptations must provide fitness advantages, otherwise they would not persist (discussed in Shettleworth, 1998). While environmentally specific adaptations are beneficial, it is unclear how many animals respond to new changes in their environments, and what ecological impacts these changes can have.

A major factor in changing environments is changing temperature. To cope with changing temperatures, birds employ a variety of responses. Some species (e.g. wood warblers) migrate to avoid unfavourable temperatures all together, while other species have adapted to cope in different ways (e.g. facultative hypothermia as mentioned above). Non-migratory species are of particular interest because these species are likely to be greatly affected by environmental change given the broad range in environmental conditions naturally experienced. Ambient temperature affects birds both behaviourally and physiologically (White et al., 2007; Chatelain et al., 2013; Taylor et al., 2014), and in recent
years both the average global temperature (Hansen et al., 2006), and the temperature in Canada (Zhang et al., 2000) have increased. These environmental changes are having clear ecological impacts on Black-capped chickadee populations. For example, warming has led to a change in the range of Black-capped chickadees, causing the northern-most limits of their populations to expand further north (Taylor et al., 2014). These trends have also impacted the hybridization zone, a zone of overlap between the southern limit of Black-capped chickadees and the northern limit of Carolina chickadees (*Poecile carolinensis*), causing a northward movement of the hybridization zone (Taylor et al., 2014). Along with these known effects, changing temperatures could have further, wide ranging, effects on Black-capped chickadees, and other avian populations and assemblages.

1.3 Behavioural and Cognitive Effects

Many animals are sensitive to a variety of environmental cues and respond to these cues in a variety of ways. Because of the breadth of responses, examples of environmental cues modulating behaviour are abundant. For example, multiple ant species change their foraging pattern seasonally, a change most likely due to seasonal differences in rainfall (Rockwood, 1975). Additionally, Japanese quail (*Coturnix japonica*) have been shown to modulate gizzard and other organ size, based on a seasonally changing diet (Starck, 1999). In chickadees specifically, previous work suggests that individuals from harsh environments benefit from enhanced cognitive abilities to deal with the greater demands of survival in harsh environments (Roth & Pravosudov, 2009; Roth et al., 2010; Kozlovsky et al., 2015). Though not explicitly stated in these studies of chickadees, harshness is assumed to be a combination of environmental factors such as temperature, altitude, precipitation, and food supply. These previous studies have sampled different populations of chickadees from different geographic locations, focusing on elevation and latitudinal differences. Roth et al.
(2010) found differences in problem solving ability and response to novelty when comparing Black-capped chickadee populations from Alaska and Kansas. These cognitive differences associated with inhabiting a harsher climate are thought to be due to genetic differences between populations (Roth et al., 2010). While this may be a partial explanation, it may not be the whole picture. There is evidence to suggest that experience (Clayton & Krebs, 1994; Clayton, 2001), or environmental complexity (Barnea & Pravosudov, 2011), can change brain morphology and it is reasonable then to suggest that experiences could influence cognitive abilities.

Problem solving ability and risky behaviours are likely useful for exploiting novel resources (Sol et al., 2011). If experience can affect behavioural and cognitive responses, then these factors could greatly affect survival in chickadee populations. As discussed previously, the home range of Black-capped chickadees’ is shifting, and as their environments change, they are more likely to encounter novel situations. Strategies to cope with these novel situations should become increasingly common, and the ability to flexibly utilize these strategies should be of the greatest benefit.

1.4 Avian Hippocampus and Food Storing

The avian hippocampus is a functional homologue of the mammalian hippocampus, and is essential for processing spatial information (Colombo & Broadbent, 2000). Spatial information, and by extension the hippocampus, is important across a broad spectrum of ecological contexts. For example, brood parasites such as brown-headed cowbirds (Molothrus ater) use spatial information to keep track of parasitized nests (Sherry et al., 1993; Reboreda et al., 1996) and pigeons have been shown to use the hippocampus for navigation (Bingman et al., 1990; Bingman et al., 2006). Perhaps the best studied example,
however, is that of food storing birds, which use the hippocampus for the accurate retrieval of caches (Sherry, 1984; Clayton & Dickinson, 1998).

Species that store food have a relatively larger hippocampus than species that do not (Krebs et al., 1989; Sherry et al., 1989), suggesting that these specialized hippocampi have valuable ecological impacts (as discussed in Shettleworth, 1998). In Black-capped chickadees, for example, the volume of hippocampus is known to change seasonally (Hoshooley & Sherry, 2004; Hoshooley et al., 2005; Hoshooley & Sherry, 2007), although the exact cause of this variation remains unknown. It is possible that this seasonal variation is in response to an increased memory load and that as caching behaviour increases, hippocampal volume increases as well. Black-capped chickadees can cache thousands of food items in a season in scattered hoards. This means that they cache a single piece of food in each of many locations, rather than many pieces of food in few locations in the way that larder hoarding species do. Black-capped chickadees can accurately remember the location and contents of their caches (Sherry, 1984; Feeney et al., 2009), and that memory for these caches can last up to a month (Hitchcock & Sherry, 1990). As a by-product of this strategy, individuals have a higher memory load and would benefit from enhanced spatial cognition, especially during the caching season. Individuals cache food at the highest rates in the fall (Smith, 1991) as they prepare for anticipated limited resources during winter. This caching behaviour can be modulated using photoperiod, with the shortening of day leading to increased caching behaviour (MacDougall-Shackleton et al., 2003). It stands to reason then, that hippocampal volume should vary with these changes in behaviour, however, this is not the case as caching behaviours can change without changes occurring in hippocampus (Krebs et al., 1995; MacDougall-Shackleton et al., 2003).
1.5 Temperature Related Shifts in Reproductive Timing in Birds

Arguably the most important phenological event for many animals is reproduction. The timing of reproduction can greatly impact offspring survival, and therefore affect individual fitness. Because of this, many species time reproduction to maximize resource availability for provisioning offspring. For example, migrating birds time their arrival on the breeding grounds, and subsequently their reproduction, to ensure peak resource abundance (Møller et al., 2010). Additionally, great tit populations also match reproductive timing with peak resource abundance (Perrins, 1991). This seasonal matching between reproductive timing and peak resource abundance clearly comes with fitness benefits, and it is possible that a mismatch in this timing would lead to severe negative effects.

These negative effects can be seen in a variety of taxa, and at a variety of trophic levels. Mismatches between calving caribou (*Rangifer tarandus*) and plant abundance in the Arctic have caused a decrease in reproductive success (Post & Forchhammer, 2008). This difference is caused by a simple mismatch between calving, which is cued by photoperiod, and plant growth, affected by a variety of environmental factors (Post & Forchhammer, 2008). As previously mentioned, birds are also experiencing similar mismatches in resource abundance and reproductive timing. Arthropod abundance is shifting earlier in the spring as the global temperature increases (Visser et al., 1998). While some research has shown that avian species are able to at least partially accommodate shifts in reproductive timing (e.g. Visser et al., 2006), other studies have suggested the opposite (e.g. Mayor et al., 2017). In either case, these results are correlational, and there is a lack of empirical evidence linking these shifts to temperature changes. More exploration is needed in order to understand how birds might be shifting reproductive timing, and what the consequences will be if species fail to adequately shift their reproductive timing.
1.6 The Current Study

This thesis examines the effects of winter temperature on three distinct aspects of the Black-capped chickadee’s annual cycle. The first experiment examines temperature effects on cognition and behaviour, the second examines temperature effects on hippocampal volume, and the third examines temperature effects on reproductive condition and reproductive readiness.

The first experiment examined cognition and behaviour. Individuals were tested on problem solving ability and responses to novel stimuli, both in a foraging context. To test problem solving, birds were presented with a food reward that was partially obstructed by a removable barrier. The problem was considered solved once the barrier was removed by the bird and the reward was obtained. To examine individuals’ responses to novel stimuli, a novel food dish containing a food reward was presented, and the latency to remove the reward was recorded. It was hypothesized that temperature would influence both problem-solving ability, and an individual’s response to novel stimuli. Results indicate, however, that temperature had no effects on problem-solving abilities, or how individuals responded to novel stimuli.

The second experiment explored the possibility that temperature could serve as an external cue for the regulation of hippocampal volume. Immediately after behavioural testing, individuals were perfused, their brains were collected, and hippocampal volume was estimated for each individual. It was hypothesized that temperature would influence hippocampal volume. Results, however, indicate that temperature does not have an effect on hippocampal volume in Black-capped chickadees, and is not responsible for the seasonal variation observed in previous studies.
The third experiment examined the effects of temperature on gonadal development and reproductive readiness. At the time of sacrifice, male testes were measured and those measurements were used to calculate testis volume. Females’ ovaries were scored both visually, for reproductive condition, and by recording ovary mass. It was hypothesized that temperature would have an effect on reproductive condition in both males and females. Both males and females that experienced warmer winters had more developed gonads. Results suggest that temperature may be a secondary cue, in addition to photoperiod, used by chickadees to time reproduction.

As the global climate continues to change, birds will increasingly be faced with novel and changing environments. The results of this thesis suggest that Black-capped chickadee populations will be able to shift their reproductive timing in order to match peak food abundance. Additionally, I found that Black-capped chickadees do not use temperature cues to modulate behaviour or hippocampal volume. Understanding how species like the Black-capped chickadee respond to these changes is essential in shaping conservation efforts. By understanding how species are responding to changing environments, policy makers will be better able to plan for the conservation of future populations.
1.7 References


White, C., Blackburn, T., Martin, G., & Butler, P. (2007). Basal metabolic rate of birds is
associated with habitat temperature and precipitation, not primary productivity.

*Proceedings of the Royal Society: Biological Sciences*, 274, 287-293.

1.8 Figures

Figure 1.1 The range of the Black-capped chickadee. The Black-capped chickadee is a small, non-migratory passerine bird, and the study species in this thesis (from *Birds of North America*, https://birdsna.org, maintained by the Cornell Lab of Ornithology).
2 Overwinter temperature, problem solving, and responses to novelty in Black-capped chickadees (*Poecile atricapillus*)

2.1 Introduction

As the cost of living in an environment increases, animals should benefit from more refined cognitive and behavioural responses (Shettleworth, 1998, Pravosudov & Clayton, 2002). Environments can be costly for any number of reasons (e.g. anthropogenic influences, changing climatic conditions). Regardless of the cause, these costs should have similar effects on individuals inhabiting the costly environments. This concept has been studied broadly, and in a variety of taxa, but perhaps most extensively in birds (reviewed in Crick, 2004). For example, it has been shown that House sparrows (*Passer domesticus*) invading urban areas have less neophobic responses to novel food sources than established birds (Martin & Fitzgerald, 2005). It has also been shown that urbanized Common mynas (*Acridotheres tristis*) inhabiting novel and complex environments are more innovative when working for a food reward when compared to rural conspecifics (Sol et al., 2011). In addition to these anthropogenic factors that modulate behaviour, it is also well documented that in populations of various species of chickadees, climatic factors influence behavioural responses. Mountain chickadees (*Poecile gambeli*) from high elevations solve problems faster than individuals from low elevations (Kozlovsky et al., 2015), and Black-capped chickadees (*Poecile atricapillus*) from northern latitudes solve problems more efficiently, and are less neophobic than their more southern conspecifics (Roth et al., 2010). Differences between populations of individuals across climatic gradients continues beyond problem solving ability and neophobic responses. Differences in food-caching ability and spatial memory have also been found between populations of Black-capped chickadees, in which
individuals from higher latitudes cache more and recover caches more accurately than individuals from lower latitude populations (Roth et al., 2011). These findings make initial intuitive sense, as individuals from harsh environments (e.g., complex landscapes, high elevation, high latitude) may more readily exploit novel or difficult food sources as a means of survival. Many of these findings suggest that selection has acted on these different populations (e.g., high elevation vs low elevation) and is responsible for the variation in behavioural responses. Recent work, however, suggests that genetic differences cannot explain such population differences in every circumstance. In a study comparing high and low elevation populations of Mountain chickadees, Branch et al. (2017) found no evidence of genetic differences in population structure. My research examines whether individual experience within a bird’s lifetime, rather than the effects of natural selection, might explain observed differences in behaviour and cognition between populations.

One of the most variable environmental experiences across an individual’s lifetime is temperature. An individual from a single population can experience a wide variety of temperatures over the course of its life. For small, non-migratory birds such as the Black-capped chickadee, winter temperature is extremely important. Populations, especially those wintering at northern latitudes, can experience great variation from one winter to the next. For example, the home range of the Black-capped chickadee experienced winter temperatures between 0.5 °C warmer and 6 °C colder in 2016 than in 2015 (NASA GISS; Figure 2.1a), and between 0.5 °C colder and 5.8 °C warmer in 2017 than in 2016 (NASA GISS; Figure 2.1b). This clear annual variation in temperature is experienced by individuals in populations, and is potentially capable of modulating behavioural responses. Temperature is known to affect foraging behaviour in birds. Chatelain et al. (2013) demonstrated that a reduction in ambient temperature will increase the predation of a known toxic prey by
European starlings (*Sturnus vulgaris*). Caraco et al. (1990) also demonstrated that
temperature influences foraging choice. In a two-choice task, Yellow-eyed juncos (*Junco phaeonotus*) in cold environments favoured a variable high-risk, high-reward option, while
birds in warmer environments opted for the constant low-risk, low-reward option.

If ambient temperature can modulate behaviour in foraging contexts it is reasonable
to believe that seasonal temperature can at least partially modulate behaviour previously
attributed to adaptive differences between populations. In this study, I examine the effects of
winter temperature on problem solving ability and individuals’ responses to novelty in Black-
capped chickadees. I hypothesized that seasonal winter temperature would have an effect on
behaviour, and predicted that birds experiencing a colder winter environment would solve
problems faster, and be less neophobic than their conspecifics that experienced a warmer
winter environment.

2.2 Methods

2.2.1 Subjects

Forty-nine Black-capped chickadees were caught between October and December of
2015 (27 birds; 8F:19M) and 2016 (22 birds; 10F:12M), on the Western University campus.
Each bird was given a unique colour band combination for identification. Birds were group
housed in large outdoor aviaries at the time of capture in flocks ranging from six to twelve
members. Individuals were moved to outdoor individual cages on January 8 of each year.
Food was provided *ad libitum*, and water was provided twice daily (approx. 9am and 2pm) to
avoid freezing in sub-zero temperatures. Mealworms and black oil sunflower seeds were
provided daily. Individuals were provided a house box (7.6 x 7.6 x 7.6 cm) with straw, cotton
and yarn for insulation.
2.2.2 Treatments and Heating

All additional heat was provided by PrimeGLO Telescopic Electric Heaters (AZ Patio Heaters, Peoria, AZ). Beginning January 15 (in each respective year), birds were assigned one of three treatment groups: Control (n=16), 6H (n=17), and 24H (n=16). Birds in the Control group experienced natural temperatures in their outdoor cages. Individuals in the 6H treatment experienced natural temperatures, but received six hours of additional heat every twenty-four hours, between 10pm and 4am. These hours allowed the birds to avoid what is typically the coldest daily temperature. Individuals in 24H experienced twenty-four hours of additional heat daily to simulate a warmer winter. The Control, 6H and 24H treatments all experienced variation in temperature. For the Control group this was normal daily variation in winter temperature, while for the 24H group it was natural variation with a warming effect superimposed (Figure 2.2). For the 6H group, temperature oscillated between the natural temperature experienced by the Control group during the day, and the warmer temperature experienced by the 24H group at night (Figure 2.2). Birds were housed in heated treatments until the completion of cognitive testing. Temperature was measured on the exterior of the house boxes (2 for Control & 6H, 3 for 24H) in all treatments using HOBO® Pendant® Data Loggers (Onset Computer Corporation, Bourne, MA).

2.2.3 Body Composition and Sex

Body composition (dry fat and wet lean mass ± 0.01 g) was measured for each individual at three time points during the experiment using a quantitative magnetic resonance (QMR) scanner (Echo-MRI-B, Echo-Medical Systems, Houston, TX, USA). Pre-Treatment measurements were taken at least one week after capture, but before birds were allocated to a treatment group. This waiting period between capture and measurement allowed birds to habituate to captivity and allowed food intake and energy expenditure to stabilize prior to
taking body composition measures. During-Treatment measurements were taken three days prior to the beginning of testing. This was done to get an accurate measure of body composition for the testing period without subjecting the birds to body composition scans on testing days. Post-Testing measurements were taken within one week of testing the final bird. Due to extended testing in year one, however, these dates differ between years. Year one birds’ Post-Testing measurements were taken in May, while year two birds’ Post-Testing measurements were taken in April. The sex of each individual was determined post-mortem by gonad inspection.

2.2.4 Behavioural Testing

Behavioural testing began on March 20th and all trials were completed in the testing individual’s home cage. All tasks were video recorded, and the tasks were completed in the order described below.

2.2.5 Video Recording and Analysis

All trials for Problem Solving I, Problem Solving II, and Response to Novelty were recorded to minimize effects of direct visual observation. Videos were recorded using one of four different camera models (GoPro® HERO4 Silver camera, GoPro Incorporated, San Mateo, CA, USA; Canon VIXIA HF R40, Canon Canada Incorporated, Mississauga, ON, Canada; Activeon CX Action Camera, Activeon Incorporated, San Diego, CA, USA; CX Gold Action Camera, Activeon Incorporated, San Diego, CA, USA). Videos were uploaded to a computer and analysed upon the completion of all trials. Videos were analysed by observers blind to the treatments. Each video was scored twice, and the mean latency for each phase (see task specific sections) from both scorings was used for analysis. The onset of a trial was marked by the testing apparatus being stationary, with the experimenter out of
the frame. In the case that the two initial latency scores were greater than 5 seconds apart, a third scoring was completed, and mean of the two most similar scores was used.

2.2.6 Problem Solving I

Problem solving was tested using a modified version of the problem-solving task in Roth et al. (2010). The problem solving task is meant to assess a bird’s ability to manipulate its environment in order to retrieve a partially obstructed food reward.

2.2.6.1 Testing Apparatus and Habituation

A wooden board (40cm x 18cm) was drilled with 15 identical wells (1.5 cm diameter; Figure 2.3). In experimental trials, a stainless-steel washer (3.5 cm diameter, 1.5 cm opening), with a clear plastic coating (Con-Tact Brand®, Kittrich Corporation, La Mirada, CA), was used to cover a single well. The covered washers allowed birds to see the food reward, but not access it without first removing the washer. Mealworms were placed in the covered well, and used as a food reward. Two days prior to testing, birds were exposed to both the board and the washer for at least 36 hours to minimize neophobic responses. The board contained mealworms in 8 of the 15 wells (at least one in each row, and column), and the covered washer was fastened adjacent to the central well such that the birds could interact with it, but not move it. Birds were allowed 24 hours to interact with both the board and the washer, and habituation was considered successful if food was retrieved from all 8 baited wells. One-hundred percent of birds successfully cleared all wells and moved on to experimental trials.

2.2.6.2 Testing and Scores

Birds were food deprived for one hour prior to testing. Testing occurred in three phases: pre-trial motivation, experimental-trial, and post-trial motivation. The three phases were performed in succession, with as little inter-trial time as possible. The pre-trial and post-
trial motivation phases were used to ensure that motivation did not vary before and after the experimental phase. The first touch score (in phase 2) was used to control for neophobic responses. Each bird was assigned a ‘rewarded well’ and that well remained the same for all three phases. In the pre-trial motivation phase, a mealworm was placed in the rewarded well, and the board was placed into the home cage of the testing individual. After 5 minutes had elapsed, the board was removed and a new mealworm was placed in the rewarded well. A washer was placed over the well, and the board was returned to the home cage of the testing individual starting the experimental-trial phase. Birds were allowed 30 minutes to retrieve the mealworm. The post-trial motivation phase followed the same procedure as the pre-trial motivation phase and immediately followed the experimental-trial. A total of four latency scores were reported for each individual. The pre-trial motivation phase score was the latency of an individual to remove a mealworm from the baited board, and was reported as the ‘pre-trial’ score. The experimental phase resulted in two scores; 1) the latency of a bird to make contact with the board or the washer was reported as the ‘first-touch’ score and 2) the latency of a bird to move the washer and remove the worm was reported as the ‘problem solving’ score. The post-trial motivation phase score was the latency of a bird to remove a worm and was reported as the ‘post-trial’ score.

2.2.7 Problem Solving II

Problem Solving II followed the same procedure as Problem Solving I, with the experimental phase extended to 1 hour. This manipulation was done in response to the low completion rate of birds in Problem Solving I, and was designed to increase the number of birds that were able to solve the task without receiving a maximum time score. Birds 28-49 were allowed this 1 hour retrieval period.
2.2.8 Response to Novelty

Testing response to novel stimuli was done in a modified version of the task described in Roth et al. (2010). This task is designed to reveal behavioural differences in responses to novelty.

2.2.8.1 Testing Apparatus and Habituation

Silver coloured food cups were used as the standard food dish. Novel food cups were created by altering the birds’ standard food dish with paint, metal nuts and metal bolts (Figure 2.4). Blue and yellow paint was added to the sides of the food bowl in dots such that one dot of each colour was always visible when viewing the dish in profile. Gold coloured nuts and bolts were attached to each dish to alter the shape of the dish when viewed from overhead. Food dishes and rewards were presented by sliding a bottom tray out of the home cage of the individual being tested, and placing the dish on the tray. The tray was then slid back into the home cage. Individuals were presented with a single mealworm in the standard food dish each day for four days in order to allow the birds to habituate to the dish.

2.2.8.2 Testing and Scores

Birds were food deprived for one hour prior to testing. Testing occurred in three phases; pre-trial motivation, experimental-trial, and post-trial motivation. The three phases were performed in succession, as quickly as possible. In the pre-trial motivation phase, birds were presented with a single mealworm in the standard food dish, and allowed 300 seconds to retrieve it. After 300 s had elapsed, the dish was removed and replaced with a novel food dish containing a single mealworm to initiate the experimental-trial phase. Individuals were allowed 900 s to complete this trial. The post-trial motivation phase was performed in the same manner as the pre-trial motivation phase. A total of three scores were reported for each individual: 1) The pre-trial motivation phase score was the latency of a bird to remove a
worm from a standard food dish, and was reported as the ‘pre-trial’ score; 2) The experimental phase score was the latency of a bird to remove a worm from a modified food dish, and was reported as the ‘experimental test’ score; 3) The post-trial motivation phase score was the latency of a bird to remove a worm from a standard food dish, and was reported as the ‘post-trial’ score.

2.2.9 Statistical Analysis

All statistical analyses were done using SPSS (version 24, IBM Corporation) unless otherwise noted. All significant interactions and main effects are reported. All interactions that are not reported were non-significant.

2.2.9.1 Temperature Treatments

To compare the temperatures between both years and temperature treatments, I used a two-way ANOVA, and performed a Tukey’s test post-hoc. Both tests were done using R (version 3.4.0, GUI 1.40, R Development Team 2017).

2.2.9.2 Body Composition

To compare body composition, a general linear model (GLM) was used. Stage of the experiment (Pre-Treatment, During-Treatment, Post-Testing) was included as a within-subjects variable, and temperature treatment (Control, 6H, 24H), sex, and year were included as between-subject variables.

2.2.9.3 Behavioural Data

For all behavioural measures, untransformed data are presented visually for clarity. All statistical analyses were done on log transformed values. To compare latencies, a GLM was used and included phase (Pre-Trial Motivation, Experimental Trial, Post-Trial Motivation) as a within-subject variable, and temperature treatment (Control, 6H, 24H), sex and year as between-subject variables. To further analyze the interaction in Problem Solving
I used a chi-square test to compare the completion percentage of each temperature treatment group across years. The GLM for Problem Solving II did not include year, as only birds from year two were tested in Problem Solving II.

2.3 Results

2.3.1 Temperature Treatments

While there were overall differences between years in mean temperature (F_{1,96}=8.60, p=0.004) and between temperature treatments (F_{2,96}=120.2, p<0.0005), multiple comparisons revealed no differences between years for the same temperature treatments (Control: p=0.95; 6H: p=0.52; 24H: p=0.13).

2.3.2 Body Composition

2.3.2.1 Fat Mass

There were no significant differences in fat mass (Figure 2.5) between temperature treatments (F_{2,46}=2.250, p=0.12), sex (F_{1,47}=3.491, p=0.07) or year (F_{1,47}=0.315, p=0.57). The During-Treatment fat mass however, was significantly higher (p < 0.005 for both comparisons) than the Pre-Treatment and Post-Testing fat mass which did not differ.

2.3.2.2 Lean Mass

Temperature treatment had no effect on the lean mass (F_{2,46}=1.627, p=0.211; Figure 2.6). Males consistently had more lean mass than their female counter parts (F_{1,47}=11.388, p=0.002). Birds from year one also had more lean mass than birds from year two (F_{1,47}=9.151, p=0.005). These differences are likely due to the sex ratio of males to females in each year (Year 1=8F:19M, Year 2=10F:12M) as males have more lean mass than females.
2.3.3 Problem Solving I

Problem solving ability was assessed by the latency to retrieve a food reward from a location that was physically, but not visually, obstructed (Figure 2.7). The problem solving phase took significantly longer (p < 0.0005 for all comparisons) than both motivation phases, which did not differ (p=1.00). While temperature treatment (F2,46=1.667, p=0.20), sex (F1,47=1.010, p=0.32), and year (F1,47=1.633, p=0.21) all had non-significant effects on problem solving, a significant interaction was found between temperature treatment and year (F2,43=5.723, p=0.001; Figure 2.8). This interaction appears to be driven by the inability of some individuals to complete the task (Figure 2.9). In year one, significantly fewer individuals in the Control group completed the task when compared to individuals in both the 6H and 24H groups (chi-square test: \( \chi^2 = 7.67, p=0.022 \)). In year two however, 100% of Control birds competed the task, while some individuals from the 6H and 24H groups failed. In year one the rank order of the means from fastest to slowest was: 6H (790.6s; 78% completion), 24H (823.5s; 78%), Control (1446.6s; 22%). In year two the rank order was; Control (255.1s; 100%), 24H (885.1s; 57%), 6H (1166.2s; 63%). This rank order change in the means is likely driving the interaction between year and treatment.

2.3.4 Problem Solving II

Problem solving ability for Problem Solving II was assessed in the same way as Problem Solving I above, but with the maximum trial time extended to 1 hour. Temperature treatment again had no effect on problem solving ability (F2,19=2.202, p=0.15; Figure 2.10). There was no sex difference between males and females (F1,20=0.460, p=0.51).

2.3.5 Response to Novelty

Response to novelty was assessed by recording the latency of individuals to eat from a novel food dish. Motivation was controlled pre- and post-trial by recording the latency to
retrieve a worm from a standard food dish, and Pre-Trial and Post-Trial Motivation did not differ (p=1.00) but did differ from the Experimental Test phase (p=0.0005). Neither temperature treatment (F$_{2,36}$=1.258, p=0.30; Figure 2.11), nor year (F$_{1,36}$=0.262, p=0.61) had an effect on individuals’ response to novelty. Males were significantly less neophobic than females (F$_{1,36}$=5.938, p=0.02)

2.4 Discussion

The temperature a bird experienced did not influence its fat mass or the amount of lean mass that an individual had. No difference in fat mass is particularly interesting because it has been proposed from theoretical considerations that temperature should be an accurate predictor of fat (Houston & McNamara, 1993), and observed empirically that the lower the temperature, the more fat an individual has (Gosler, 1996). It is worth noting that in all three temperature treatments, fat mass increased from Pre-Treatment to During-Treatment. This is consistent with many observations from the field (e.g. Petit & Vézina, 2014; Petit et al., 2014) and in the wild is likely due to the unpredictability of food, along with energetically taxing cold nights. There are several possible reasons that the control, 6H and 24H birds did not differ in fat mass. First, it is possible that the time of day the measurements were taken affected fat mass. Many small birds, due to their a high metabolic rate, cannot carry more fat than will sustain them for about 1-2 days (King, 1972). As a result, many of these species, including the Black-capped chickadee, undergo a significant daily variation in fat mass, with stores being depleted overnight and replenished during the day (Baldwin & Kendeigh, 1938; Chaplin, 1974). Because all body composition measurements were taken in the morning, it is possible that differences between treatments that emerge over the course of the day were not yet apparent. If the birds had been measured later in the day, the fat score might have been more indicative of the maximum fat mass of each individual. If this idea is correct, then it is
possible that the data collected better represent the rate of fat replenishment in each treatment. As all individuals would have just used their fat stores through the night, the fat they had when measured would just be the fat they had replenished in the time before measurements were taken. Birds in all treatments were allowed between 1-2 hours of food access before being measured, and individuals were sampled in a pattern such that the mean access to food should be roughly equal among groups (Control, 6H, 24H, 24H…). It is possible then, that there are no differences in the replenishing rate of fat stores but the maximum value of the fat stores, immediately before nightfall, would be different between groups.

Temperature treatment did not have a significant effect on either problem-solving abilities or individuals’ responses to novel stimuli. These findings provide no evidence that winter temperature drives changes in problem solving abilities and responses to novelty in Black-capped chickadees. These findings do, however, provide evidence of the scope of individual variation among a genetically similar population, and provide evidence that behavioural responses can change from year to year. It is also worth noting that the variation in scores between treatments, and between years, might not entirely exclude experiential effects and support the conclusion that differences between populations experiencing different harshness as previously hypothesized (e.g. Roth et al, 2010). The interaction in Problem Solving I, where the rank order of the treatment means was reversed from year one to year two, suggests that there may be other factors, not controlled or tested in this study, that can modulate behavioural responses. Some potential factors can probably be discounted because they are linked in some way to temperature. For example, birds are known to be sensitive to humidity (e.g. Grubb, 1975; Gerson & Guglielmo, 2011), but relative humidity depends greatly on temperature and so it would not adequately explain the variation in
behaviour observed. Other variables cannot be discounted so easily. For example, social complexity could potentially modulate behaviour, and problem solving abilities. Social structure and social complexity can influence behaviour in multiple species of chickadee (Smith, 1991; Gosler, 1996; Freeberg, 2006). Individuals could potentially have had different levels of interaction with other birds. While no birds were visually or acoustically isolated from other birds in captivity, it is possible that there was a difference in proximity to other birds. These differences could lead to differences in signal intensity and overall social complexity. It was also impossible to control visits by wild birds to the exterior of the enclosure. Any of these factors could alter the social complexity of an individual’s environment, and by extension, alter problem solving behaviour and responses to novelty.

In general, the findings of this study suggest that behavioural and cognitive differences previously observed in Black-capped chickadees are not due to plastic responses in response to winter temperature. If differences between local populations are the outcome of natural selection and not individual flexibility, the inability of individual birds to adapt to rapidly changing conditions could be detrimental to their continued survival (Gaston, 2003). As the global climate continues to change, the ability to exploit changing resources will become increasingly important. This knowledge can be used to influence future conservation efforts and policy decisions. While the Black-capped chickadee is not specifically a species of concern, the idea that even such behaviourally flexible animals may be unable to adapt individually to changing environmental conditions should be particularly eye opening, and potentially cause for increased concern.

2.5 Conclusion

Behavioural differences have been well documented in chickadee species from different environments. The majority of these differences have been attributed to genetic differences
resulting from long term selection on these populations. Although new evidence suggests that these genetic differences may not be present in every case (Branch et al., 2017), the hypothesis that genetic differences are still responsible for behavioural differences is the best supported hypothesis to date. In this study, I tested experiential effects on many of the same traits examined in previous studies. My results suggest that short term, overwintering experience does not affect problem solving abilities, or an individual’s responses to novel stimuli. These results lend indirect support to the aforementioned genetic hypothesis.

Understanding what drives behavioural responses in natural populations is essential in advancing our understanding of animal behaviour in the wild, and can influence conservation and policy decisions moving forward.
2.6 References


GIStEMP Team, 2016: *GISS Surface Temperature Analysis (GIStEMP)*. NASA Goddard


Pravosudov, V. V., & Clayton, N. S. (2002). A test of the adaptive specialization hypothesis:


2.7 Figures

Figure 2.1 Heat maps showing the change in mean winter temperature (Dec-Feb) between a) 2016 and 2015, and b) 2017 and 2016. Range of the Black-capped chickadee outlined in black (from GISTEMP Team).
Figure 2.2 Mean temperature recordings from 2016 are shown for each of the treatments as an example. The red line is the temperature of the cages that were provided 24H of additional heat, the blue line is the temperature in cages that were provided 6H of additional heat, and the black line is the temperature of Control cages. The 24H cages were always warmer than birds kept at Control temperatures, and the 6H group oscillated between Control (during non-heating hours) and 24H (during heating hours) temperatures.
Figure 2.3 For the problem solving task, this board was presented to an individual.

In this photo, well B-3 is the rewarded well. The washer has been placed over the food reward in the well, and must be moved by an individual before the reward can be accessed.
Figure 2.4 Photos of the standard (left) and modified (right) food dishes used in the response to novelty task. Modified dishes were created by adding yellow and blue paint, as well as gold coloured nuts and bolts to a standard food dish.
Figure 2.5 Fat mass did not differ between temperature treatments at any of the three measurement points. Measurements were taken before, during and after the heating treatments were applied. The black line is the median value, the coloured boxes contain the 25\textsuperscript{th} and 75\textsuperscript{th} percentiles, and the dashed lines are the maximum and minimum values. Dots above the maximum value are outliers.
Temperature treatment had no effect on lean mass at any of the three measurement points. Measurements were taken before, during, and after the heating treatments were applied.
Figure 2.7 Problem Solving I consisted of three phases, with phase 2 resulting in 2 scores. Pre-Trial and Post-Trial Motivation scores were used to ensure that all birds were motivated throughout the duration of the test. The First Touch score was used to ensure the apparatus did not elicit a neophobic response from the birds, and the Problem Solving score was used to assess problem solving abilities. The red horizontal line indicates that maximum trial time. Data shown are combined data from both years.
Figure 2.8 Year had an effect on how temperature treatment influenced individuals in each treatment group. In year one control birds had very long latencies compared to both the heated treatments, while in year two, the Control birds completed the task the fastest. Birds in the heated treatments showed large variation in both years. The red line indicates the maximum trial time allowed.
Figure 2.9 Percentage of individuals that completed the task in each temperature treatment group in each year. The Control treatment had the biggest difference between years because only 22% of birds completed the task in year one, but 100% completed the task in year two.
Figure 2.10 There were no significant differences in *Problem Solving II* between the temperature treatments. While Control birds completed the task the fastest, the groups overlap considerably in latency. The red line indicates the maximum trial time.
Figure 2..11 Response to novelty was tested using a three-phase design. The left and right panels represent the Pre-Trial and Post-Trial Motivation phases, and the center panel represents the Experimental Trial phase in which the latency to remove a reward from a novel food dish was scored.
3 Temperature has no effect on hippocampal volume in Black-capped chickadees (*Poecile atricapillus*)

3.1 Introduction

Most animals undergo some degree of seasonal change, and this is especially true for birds. Examples of these changes can occur on a variety of scales, from hormonal changes (e.g. testosterone; Farner, 1973) to behavioural changes (e.g. nest building behaviours; Collias & Collias, 1984), to morphological changes (e.g. plumage; Holmgren & Hedenström, 1995). In many passerines, some of the most notable seasonal changes occur in the brain. For example, canaries have larger song control nuclei, the portion of the brain responsible for song production, in the spring when they are actively singing, than in the fall, when they are not (Nottebohm, 1981). This seasonal plasticity is an essential characteristic of many species that inhabit seasonal environments, allowing individuals to cope with seasonal differences throughout the annual cycle (Tramontin & Brenowitz, 2000).

While often studied in the breeding season, seasonal plasticity can occur at other times of year too. Food storing species, like Black-capped chickadees, hoard and retrieve food as a strategy to ensure a consistent food source through unfavourable or costly climatic conditions. This behaviour is most concentrated during the fall and winter months, when food abundance can be unpredictable (Smith, 1991). Past research has also shown that Black-capped chickadees undergo seasonal structural and neurological changes in the hippocampus (Barnea & Nottebohm, 1994; Smulders et al., 1995), the region of the brain involved in memory for cache sites (Sherry & Vaccarino, 1989; Sherry et al., 1989). While much is known about the timing of these changes from direct observation and laboratory experiments, there is still investigation needed into the regulatory cues of these changes.
Much of the avian annual cycle is cued, at least in part, by photoperiod (reviewed in Dawson et al., 2001). For example, photoperiod, in combination with other proximate cues, has been shown to drive neurological changes in song control systems (Smith et al., 1997), regulate migratory timing (Berthold, 1996), and regulate breeding timing (Lofts & Murton, 1968). It makes sense then, to assume that changes to the hippocampus in food storing species would be under photoperiodic control as well. A variety of studies have tested this idea both directly, and indirectly, but with negative results (e.g. Hoshooley et al., 2005). A series of studies assessing hippocampal volume and neurogenesis, returned results with different peak volumes and rates of neurogenesis even though the study site was the same or similar (Barnea & Nottebohm, 1994; Smulders et al., 1995; Hoshooley & Sherry, 2004; Hoshooley et al., 2007; Hoshooley & Sherry, 2007; Figure 3.1). If these neural changes were in fact under photoperiodic control, the peak volume and neurogenesis rates should have been much more synchronized between years, due to the lack of variation in photoperiod. In addition to this indirect evidence that photoperiod is not the primary driver of hippocampal change, MacDougall-Shackleton et al. (2003) found no evidence that photoperiod affects hippocampal volume when imposed as a direct manipulation.

This lack of an obvious causal cue prompts the investigation into other possible cues. In this study, I tested the effects of winter temperature on hippocampal volume. I hypothesized that winter temperature would have an effect on hippocampal volume, as temperature changes are an environmental cue that should be associated with changes in resource availability. I predicted that individuals that experienced colder temperatures would have, on average, a greater winter hippocampal volume than individuals that experienced warm conditions.
3.2 Methods

3.2.1 Subjects and Specimens

17 Black-capped chickadees (13M:4F) were caught in the fall of 2015 on the Western University Campus. Birds were group housed in outdoor aviaries from the time of capture until January 8\textsuperscript{th}, 2016. The birds were then moved into outdoor individual cages containing a house box (7.6 x 7.6 x 7.6 cm) with straw, cotton and yarn for insulation. At all times food and water were provided \textit{ad libitum}, and mealworms and black oil sunflower seeds were provided daily. Beginning on January 15\textsuperscript{th}, 2016 the birds were randomly assigned to one of three treatment groups: Control (n=7), 6 hour (n=5) or 24 hour (n=5). The Control group experienced the natural winter temperature, with no additional heat provided. The 6H and 24H groups also experienced natural winter conditions, however, these groups were provided with 6 and 24 hours of additional heat respectively. All heat was provided by PrimeGLO Telescopic Electric Heaters (AZ Patio Heaters, Peoria, AZ) and recorded using HOBO\textsuperscript{®} Pendant\textsuperscript{®} Data Loggers (Onset Computer Corporation, Bourne, MA). The heating treatment continued until the birds were sacrificed.

3.2.2 Tissue Collection and Processing

Between May 24\textsuperscript{th} and May 26\textsuperscript{th}, birds were anesthetized using isoflurane and perfused with phosphate buffered saline (PBS) and 4% paraformaldehyde. Brains were dissected from the skull and stored in 4% paraformaldehyde for a minimum of 24 hours. Brains were transferred to 30% sucrose solution for a minimum of 48 hours to cryoprotect the tissue, and then frozen using crushed dry ice. Brains were stored at -80\textdegree\textsuperscript{C} until processing. Birds were sexed post-mortem by gonad examination.

Brains were sectioned coronally (40 \textmu m) and every 10\textsuperscript{th} slice was collected in three sequential series. A total of 24 sections were collected for each series. Series were
processed, and mounted on Superfrost Plus glass slides (VWR), and left to dry for a minimum of 48 hours before being coverslipped.

The primary series was stained to visualize the protein doublecortin (DCX) following the procedure of Hall et al. (2014). The tissue was washed twice in PBS (pH 7.5) for 5 min with agitation before being incubated in 30% H$_2$O$_2$ for 15 min, followed by two more rinses in PBS. Next, the tissue was then incubated in 10% Normal Horse Serum (Vector Laboratories) in 0.3% Triton X-100 (Sigma) for 1 h at room temperature with agitation. Tissue was incubated in stock DCX (C-18) primary antibody 1:250 in in 0.3% Triton X-100 (Sigma) overnight at 4 °C. The following day (minimum 12 hours later), tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation. Tissue was incubated in biotinylated secondary antibody Horse Anti-Goat IgG 1:400 with 0.3% PBS/T for 1 h at room temperature with agitation. Next, tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation before incubation in ABC Elite avidin-biotin horseradish-peroxide complex (Vector Laboratories) 1:200 with 0.3% PBS/T for 1 h at room temperature with agitation. Tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation before it was reacted (min 130 s, max 181 s) with 0.04% diaminobenzidine solution (Sigma) to visualize antibody-avidin-biotin complexes before being rinsed 4 times with PBS. Slides were dehydrated in a series of graded alcohol concentrations, cleared in xylene before being coverslipped.

3.2.3 Hippocampal Volume Calculation

Hippocampal volume was estimated by quantifying the area of the hippocampus in images of 40 μm sections of the brain and calculated the volume of the 3D structure by adding the measured areas and the calculated frustum volumes. Images of DCX labelled slides were taken using a Leica DFC420 C camera mounted on a Leica DM5500 B microscope. The hippocampus was defined as in previous work (Sherry, 1989; 2015). The
dorsal and ventral boundaries of hippocampus were defined by the dorsal surface of the brain and the ventricle respectively. The medial boundary of hippocampus was defined by the midline, and the lateral boundary was defined by an increase in cell density and variation in the size of neurons. This increase in density and size variation is characteristic of the hyperpallium apicale. Area was calculated by tracing hippocampus in each image using ImageJ software (NIH).

To estimate accurately the volume of hippocampus, the frustum volume between each area was calculated using the following formula (previously used in Sherry et al., 1989):

\[ v = \frac{1}{3}(h_1 + \sqrt{h_1 \cdot \sqrt{h_2} + h_2}) \]

where \( I \) is the distance between measured surface areas (usually 400 \( \mu m \)) and \( h_1 \) and \( h_2 \) are the measured hippocampal areas. Where sections were missing, \( I \) was adjusted. Frustum volumes were summed to estimate the total hippocampal volume.

3.2.4 Statistical Analysis

A two-way ANOVA was used to compare hippocampal volume to treatment (Control \( n=7 \), 6H \( n=5 \), 24H \( n=5 \)) and sex (male \( n=13 \), female \( n=4 \)). Statistical tests were done using R (version 3.4.0, GUI 1.40, R Development Team 2017).

3.3 Results

All hippocampal volume measurements fell between 19.14 mm\(^3\) and 29.78 mm\(^3\). The mean volume for each group was; control, 22.05 mm\(^3\); 6H, 22.91 mm\(^3\); 24H, 24.46 mm\(^3\).

Temperature treatment did not have a significant effect on hippocampal volume in Black-capped chickadees (\( F_{2,12}=1.180, p=0.34 \); Figure 3.2). There was no sex difference between males and females (\( F_{1,12}=0.03, p=0.87 \); Figure 3.3).
3.4 Discussion

My results suggest that temperature has no detectable effect on hippocampal volume in Black-capped chickadees, though there may be a slight non-significant trend towards larger hippocampal volumes in the warmer conditions (Figure 3.2). These results suggest that further investigation is required to explore temperature and other potential regulatory cues of seasonal hippocampal change.

It is possible that the non-significant trend is indicative of an effect that was missed due to sampling date. Birds were sacrificed in May, and as a result, it is possible that a difference between groups was present earlier in the year, but decreased by the sacrifice date. If this is true, then the effect that was present earlier in the year was the opposite of that predicted by previous work. Hippocampal volume should increase with a decrease in temperature as a decrease in temperature should signal increased caching behaviour (Hampton et al., 1995), and therefore, any residual effects should reflect this predicted trend. The trend that resulted from this study suggests the opposite. It is also possible that the study results reflect the beginning of a new trend. Hippocampal volume is often studied in the fall and winter as the demand for food caching increases. It is possible, however, that hippocampal volume changes in the summer as well, and for some reason warmer winter temperatures lead to greater summer hippocampal volumes. A third possibility is that the trend seen in the results of this study is due to random variation. It is impossible to assess any of these explanations without further experimentation.

While temperature does not appear to be driving changes in hippocampal volume, animals must have some kind of regulatory mechanism for if and when to increase hippocampal volume. It seems likely that some environmental cue should prompt this change, as hippocampal volume has in the past been correlated with caching behaviours, but
environmental cues are not the only cues possible. In the same experiment that showed that photoperiod had no effect on hippocampal volume (MacDougall-Shackleton et al., 2003), photoperiod did affect caching behaviour. This suggests that perhaps while increased caching and increased hippocampal volume are often correlated (e.g. Krebs et al., 1989), the relationship may not be causal. The hippocampus in Black-capped chickadees, and other birds, is largely responsible for spatial memory (Hampton & Shettleworth, 1996). Spatial memory, although important for food storage and retrieval (Sherry, 1984), is important for other activities that could be better predictors of hippocampal volume changes. Speculating with any certainty on which of these possible predictors affects hippocampal volume is difficult, as spatial memory is useful in a wide variety of contexts (Emery et al., 2007). For example, spatial memory has been shown to play a role in everything from routine foraging trips in Rufous hummingbirds (Selasphorus rufus; Hurly, 1996), to nest site recognition in brood-parasitic brown-headed cowbirds (Molothrus ater; Guigueno et al., 2016; Reboreda et al., 1996; Sherry et al., 1993). It is possible that Black-capped chickadees are using the hippocampus for tasks other than food caching, and that some other context, or a combination of contexts are required for hippocampal variation.

3.5 Conclusion

This study found no effect of temperature on hippocampus. However, the mean hippocampal volume of birds held in 6H and 24H conditions are slightly greater than those held in control conditions, though not statistically significantly. It is possible that these slight differences in groups was influenced by sampling date. It is also possible that while hippocampal variation is correlated with caching behaviour, the link is not causal. Further investigation is required to better understand the causal mechanisms of seasonal hippocampal variation in Black-capped chickadees.
3.6 References


location but not color in passerine birds. Behavioral Neuroscience, 110, 831.

Evolutionary Ecology, 9, 354-368.


MacDougall-Shackleton, S. A., Sherry, D. F., Clark, A. P., Pinkus, R., & Hernandez, A. M.


3.7 Figures

Figure 3.1 A schematic representation of variation in seasonal timing of neural changes in the hippocampus of Black-capped chickadee populations from a series of studies performed between 1994 and 2007. Letters indicate months of the year. Each line represents a different study, though some studies provided data for both hippocampal size and hippocampal neurogenesis. Image created by David F. Sherry. (Barnea & Nottebohm, 1994 (5); Smulders et al., 1995 (1); Hoshooley & Sherry, 2004 (2,7); Hoshooley et al., 2007 (3,8); Hoshooley & Sherry, 2007 (4,6))
Figure 3.2 There is no difference in hippocampal volume among treatment groups. The range of hippocampal volumes falls between 19.14 mm$^3$ and 29.78 mm$^3$. The mean volume for each group was: Control, 22.05 mm$^3$; 6H, 22.91 mm$^3$; 24H, 24.46 mm$^3$. 
Figure 3.3 Sex had no effect on hippocampal volume in Black-capped chickadees. The mean volume for each group was; males, 23.41 mm$^3$; females, 23.30 mm$^3$. 
4 Temperature regulates reproductive readiness and breeding condition in Black-capped chickadees (*Poecile atricapillus*)

4.1 Introduction

The regulation of seasonal behaviours is unequivocally important for many non-migrant species overwintering at northern latitudes. One behaviour that needs to be carefully regulated is reproductive timing. At northern latitudes, the breeding season is short, and therefore the timing of reproductive events must be precise. Photoperiod is the general regulator of annual timing in birds (as reviewed in Dawson et al., 2001), however, it has been noted that other factors are needed for more fine scale regulatory control (Wingfield, 1980).

Many species time reproduction so that breeding occurs when important resources, such as food for their offspring, are in peak abundance (Lack, 1968; Martin, 1987). For example, Great tits (*Parus major*) synchronize their reproductive timing to correspond with peak abundance of caterpillars, their main food source for feeding nestlings (Perrins, 1991). Small shifts in reproductive timing can have great ecological impacts on avian species. There is evidence that insect abundance is closely related to seasonal temperature (Visser et al., 1998), and if insectivorous species fail to accommodate shifts in prey abundance, there is the potential for a mismatch between prey availability and nestling feeding. Black-capped chickadees would benefit from the ability to fine-tune laying dates, to synchronize with peak food abundance.

Like most avian species, photoperiod controls the annual rhythm of Black-capped chickadees. Photoperiod has been used to manipulate seasonal change in the song-control system (MacDougall-Shackleton et al., 2003a), as well as food storing behaviours (MacDougall-Shackleton et al., 2003b). Photoperiod has also been used to manipulate...
reproductive condition, and reproductive condition has been used to validate photoperiod treatments (e.g. MacDougall-Shackleton et al, 2003b). Temperature, however, has never been manipulated to examine its potential effects on reproductive condition. It has been suggested that birds are able to track temperature changes and adjust their breeding timing accordingly (Hinsley et al. 2016; Phillimore et al., 2016) and this plasticity in laying date has been recently demonstrated specifically in tits. Glądalski et al. (2016) found that populations of Great tits and Blue tits (Cyanistes caeruleus) in Poland changed their lay date in response to changes in temperature. Källander et al. (2017) found similar trends in Marsh tits (Poecile palustris). Despite these results no study has directly tested the effects of temperature on breeding condition. In this study, I examined the effects of temperature on reproductive condition in both male and female Black-capped chickadees. I hypothesized that temperature would influence reproductive condition. In order to maximize fitness, Black-capped chickadees experiencing a warmer winter would be expected to enter reproductive condition sooner than birds experiencing colder temperatures.

4.2 Methods

4.2.1 Subjects

A subset of birds described in chapter 2 (birds 28-37 and 39-49; caught in 2017) were used to assess reproductive condition at the time of sacrifice (as described in Chapter 3). Individuals were sacrificed over two days (April 5/6) early in the spring, and the gonads of each individual were measured. Birds from all three temperature treatment groups (as described in chapter 2) were sampled (Control n=7, 6H n=7, 24H n=7), and all three groups contained males (Control n=4, 6H n=5, 24H n=3) and females (Control n=3, 6H n=2, 24H n=4).
4.2.2 Reproductive Condition of Males

Reproductive condition of males was scored by calculating the volume of the testes at the time of sacrifice. Digital calipers were used to obtain a maximum length and width of the left testis of each individual. Volumes were then calculated using the formula for the volume of an ellipsoid. Males were sorted into breeding (testis volume > 20 mm$^3$) and non-breeding (testis volume < 20 mm$^3$) groups based on testis volume (Phillmore et al., 2006).

4.2.3 Reproductive Condition of Females

Reproductive condition of females was assessed by visual examination of the ovaries and by recording the mass of the ovaries. Ovaries were visually scored using the following 5-point scale, adapted from MacDougall-Shackleton et al. (2001; Figure 4.1): 1, smooth with no visible follicular development; 2, granular appearance; 3, small, uniform follicles apparent; 4, follicles apparent with evident hierarchy; 5, large yolky follicles. Once visually scored, the ovaries were dissected out and the mass was recorded (± 0.1 mg).

4.2.4 Statistical Analysis

Reproductive condition was compared for both sexes independently. For males, a one-way ANOVA was used to compare testis volume among temperature treatments. Tukey’s post-hoc analyses were conducted to examine differences between treatments. Reproductive condition (breeding vs non-breeding) was analysed using a Pearson’s chi-squared test. For females, ovary development scores were analysed using a Kruskal-Wallis analysis of variance and differences between groups were examined using Dunn’s Test post-hoc and a Bonferroni correction was applied (Zar, 2010). In addition, a one-way ANOVA was used to compare ovary mass among temperature treatments. All statistical tests were done using R (version 3.4.0, GUI 1.40, R Development Team 2017).
4.3 Results

4.3.1 Male Reproductive Condition

Temperature treatment had a significant effect on testis volume in males ($F_{2,9}=17.7$, $p=0.0008$; Figure 4.2), with males receiving additional heat having larger testis volume. The Control group differed from both the 24H group (Tukey’s: $p=0.0017$) and the 6H group ($p=0.0014$). The 24H group and the 6H group did not differ ($p=0.86$).

Temperature had a significant effect on reproductive condition of males ($\chi^2=12$, df=2, $p=0.002$; Figure 4.2). 100% of birds that received additional heat, regardless of whether they were in the 6H or 24H group, were in breeding condition, while none of the Control birds were in breeding condition.

4.3.2 Female Reproductive Condition

Temperature did not have a significant effect on ovary mass in females ($F_{2,6}=2.287$, $p=0.18$; Figure 4.3), but did have a significant effect on visual ovary development score ($\chi^2=6.5$, df=2, $p=0.039$; Figure 4.4). Birds in the 24H treatment were more reproductively advanced than birds in the Control condition (Dunn Test: $Z=2.54$, $p_{adj}=0.034$), while the birds in the 6H group were not significantly different from either the 24H ($Z=0.85$, $p_{adj}=1.00$) or the Control ($Z=1.58$, $p_{adj}=0.34$) groups.

4.4 Discussion

In both male and female Black-capped chickadees, gonad development was accelerated in individuals that experienced a warmer winter but males and females were not affected equally. Individuals that received additional heat, either 6H or 24H, had larger testis than birds that received no additional heat. Also, all the males that received additional heat were in breeding condition, while birds that experienced natural conditions were not. In females, although there was no difference in ovary mass, birds that received 24H of additional heat
were in a more advanced breeding state than those individuals that experienced natural temperature conditions. Unlike the males however, none of the females were in full breeding condition.

Seasonality of breeding behaviour in birds is typically under photoperiodic control, although other factors can have an influence (Dawson et al., 2001). The current findings, however, suggest that breeding condition can be controlled by temperature, independently of photoperiod, in Black-capped chickadees. While shifts in breeding time have been correlated with temperature in some species (e.g. Visser et al., 2006), this is the first direct evidence of temperature regulating reproductive condition to my knowledge. This does not imply that photoperiod is not important in regulating reproduction in Black-capped chickadees, but that in addition to the photoperiodic cue, environmental temperature can advance or delay the onset of reproductive condition. Temperature influences on reproduction have many potential implications for breeding populations of Black-capped chickadees which may be detrimental, beneficial, or both.

Black-capped chickadees rely on insect prey to feed their young during the spring (Smith, 1991), and therefore benefit from matching their reproductive timing with peak abundance of insect prey. Relatives of the Black-capped chickadees, Great tits in northern Europe have not demonstrated the ability to flexibly alter reproductive timing to synchronize egg laying with peak caterpillar abundance (Visser et al., 1998), although there is evidence that a shift in lay date is occurring (Visser et al., 2006). If temperature can regulate reproductive readiness in Black-capped chickadees, it is possible that Black-capped chickadees are able to flexibly modulate their reproductive timing, and therefore may be better able to better synchronize their laying date with peak food abundance.
It is also possible that temperature modulation of reproductive condition could create an intersexual reproductive mismatch in breeding pairs. Although both sexes appeared to respond to warmer winter conditions with advanced reproductive readiness, on a finer scale males and females did not respond in the same way. Males that experienced warmer winter conditions (either 6H or 24H) were in full breeding condition. No females were in full breeding condition, although most were progressing toward some stage of egg production. Increased environmental temperature may affect the reproductive readiness of the sexes differently.

Also of interest is the distinct dichotomy in male bird’s reproductive condition. Males that experienced additional heat, and birds that experienced heat only overnight (6H), were in the same reproductive state. This implies that if temperature is an important regulator of reproductive readiness, the critical period is overnight. This hypothesis is of particular interest because Black-capped chickadees are known to employ facultative hypothermia to survive cold winter nights (Grossman & West, 1977). Facultative hypothermia is a strategy used by some avian species to combat low temperatures and involves lowering the core body temperature to reduce metabolic costs (reviewed in McKechnie & Lovegrove, 2002). While facultative hypothermia has been observed to decrease metabolic costs in Black-capped chickadees (Chaplin, 1974), my results suggest that this survival strategy might come with the associated cost of delayed gonadal development. If, by avoiding the use of facultative hypothermia, chickadees are able to allocate resources to gonadal development, these individuals could see an increase in fitness due to an earlier onset of reproduction and potentially a longer breeding season. It is also possible that these individuals would be better able to match reproduction to other environmental factors such as peak food abundance, that are correlated with temperature.
Females did not demonstrate the same clear dichotomy in breeding condition shown by males. This finding, however, may be limited by sample size. Of the three females that experienced warming only at night, two were in the same reproductive stage as the females that experienced 24H of additional heat. With an increased sample size, a similar dichotomy to that seen in males might be observed in females. The idea that facultative hypothermia has associated costs unrelated to immediate survival is an intriguing question that warrants further research.

4.5 Conclusion

This study has provided direct evidence that breeding condition is at least in part under temperature control in Black-capped chickadees. Temperature affects reproductive condition in both male and female birds, although potentially differently. Overnight temperature seems to be a driving factor behind reproductive readiness, especially in males, and it is possible that this is due in part to facultative hypothermia use or the lack thereof. Temperature as a regulator of breeding timing could have many ecological impacts both in relationships between species (e.g. food webs) and within species (e.g. intersexual mismatches), and as such should be further explored. The idea that facultative hypothermia, an essential strategy for survival, might have associated reproductive costs is also an area of research that warrants further investigation.
4.6 References


4.7 Figures

Figure 4.1 A visual representation of the chart used to score female reproductive condition ranging from 1, not reproductive condition to 6, intact egg. For this study birds were scored from 1-5. Adapted from the scale described in MacDougall-Shackleton et al. (2001).
Figure 4.2 Testis volumes for all three temperature treatment groups (n=4, 5, 3) with the breeding condition threshold indicated by the dotted line. Birds in both the 6H and 24H group had significantly larger testis volume than birds in the Control group. 100% of birds in the 6H and 24H group were in breeding condition, while none of the control birds were in breeding condition.
Figure 4.3 Mass of ovaries for females in each temperature treatment group (n=3, 2, 4).

There are no significant differences between groups.
Figure 4.4 Proportion of female birds at each ovarian developmental stage at the time of sacrifice (n=3, 3, 4). 24H birds were significantly more reproductively advanced. All Control birds were scored at stage 3, while all the 24H birds were scored at stage 4. One 6H bird was scored at stage 3, while the other 2 birds were scored as stage 4.
Conclusions: Winter warming affects the onset of reproduction but not cognition or the hippocampus in Black-capped chickadees (*Poecile atricapillus*)

5.1 Overview of thesis

Changing winter temperatures are likely to affect avian populations, but the extent of these effects is not known. The goal of this thesis was to further our understanding of how winter temperature may affect avian populations, using Black-capped chickadees as a model species for other resident birds. Previous research has shown that climatic conditions can affect chickadee populations’ cognition and behaviour (e.g. Roth et al., 2010; Koslovsky et al., 2015), however, the experiential effects of temperature had never been directly tested. Previous research has also demonstrated seasonal plasticity in the Black-capped chickadee hippocampus (e.g. Smulders et al., 1995), though the timing and even the occurrence of this plasticity is variable (e.g. Hoshooley et al., 2007). Photoperiod has been investigated as a potential cue for seasonal change in the avian hippocampus and was found to have no effect (MacDougall-Shackleton et al., 2003). Temperature, however, has never been investigated as a potential cue for changes in the hippocampus. Lastly, there have been recent studies demonstrating that breeding timing is plastic and can be altered by spring temperatures (e.g. Glądalski et al., 2016; Hinsley et al., 2016; Phillimore et al., 2016; Källander et al., 2017), but the direct effects of temperature on reproductive condition have never been studied.

In this thesis, the effects of overwintering temperature were tested on a population of Black-capped chickadees. The effects of temperature were studied as they related to cognition and behaviour, hippocampal volume, and reproductive timing. Temperature was manipulated by providing additional heat to some birds, but not others. Additional heat was
measured in hours/day. There were two heated treatments, and a control. The warmest treatment received 24h/day of additional heat, a second heated group received 6h/day of additional heat exclusively at night and the control group received no additional heat. These temperature manipulations had effects on reproductive timing, but not cognition and behaviour, or hippocampal volume.

5.1.1 Overwinter temperature, problem solving, and responses to novelty in Black-capped chickadees (*Poecile atricapillus*)

Previous studies have shown that environmental variables can have effects on cognition and behaviour in Black-capped and Mountain chickadees (Roth et al., 2010; Roth et al., 2011; Kozlovsky et al., 2015). Missing from this work, however, is exploration of the possible effects these same variables can have within an individual’s life. In this study (Chapter 2), I tested the effects of overwintering temperature on cognition and behaviour in Black-capped chickadees. Problem solving ability was used to assess cognition, and a response to a novel stimulus was used to assess behavioural effects. Overwinter temperature did not have any significant effects on cognition or behaviour. The reversal of the relative mean problem solving scores for each treatment, between years in Problem Solving I suggests that additional variables might be affecting cognition, and this possibility warrants exploration. In general, this study provides no evidence that overwinter temperature has an effect on behaviour and cognition, and provides further indirect evidence that population level differences in cognition and behaviour that have been previously documented in chickadees likely have a genetic basis.
5.1.2 Temperature has no effect on hippocampal volume in Black-capped chickadees (*Poecile atricapillus*)

Black-capped chickadees have been shown to undergo seasonal changes in the hippocampus (e.g. Barnea & Nottebohm, 1994; Smulders et al., 1995), however, the causes of these changes remain unknown. Previous work has provided evidence that photoperiod, a major driver of avian annual cycles (as reviewed in Dawson, 2001), is not responsible for hippocampal changes (MacDougall-Shackleton et al., 2003), and this work is supported by numerous field studies (Hoshooley & Sherry, 2004; Hoshooley et al., 2005; Hoshooley et al., 2007; Hoshooley & Sherry, 2007). I tested the effect of temperature on hippocampal volume (Chapter 3). Hippocampal volume was estimated by tracing hippocampal surface area on images of brain sections labeled for DCX expression. No effect of temperature on hippocampal volume was found. There was, however, a non-significant trend towards greater hippocampal volumes in birds that received more heat. This research suggests that, like photoperiod, temperature is not a driver of hippocampal volume variation in Black-capped chickadees.

5.1.3 Temperature regulates reproductive readiness and breeding condition in Black-capped chickadees

Modulating reproductive timing in songbirds is essential to match reproduction with peak resource availability. In this experiment (Chapter 4) I tested the effects of overwinter temperature on reproductive timing. As a measure of reproductive timing, the reproductive condition of both males and females was analyzed. Birds that received additional heat had significantly larger gonads than birds that did not receive additional heat. Because increased spring temperature has been previously linked to early peak food abundance (Visser et al., 1998), it is notable that birds which experienced warmer overwinter temperatures had more
developed gonads than birds that experienced colder temperatures. To the best of my knowledge, this research provides the first direct evidence that overwinter temperature can influence reproductive condition in an avian species. This result provides the first direct evidence that temperature can modulate reproductive timing in birds.

5.2 General Discussion

The results of my thesis provide novel insight into the effects that winter temperature can have on Black-capped chickadees. These results also reveal several possible implications, discussed below, both for Black-capped chickadee populations and for the future of research in this field. Black-capped chickadees are small passerines that are forced to deal with harsh climatic conditions because they are non-migratory, and dealing with these conditions leaves them vulnerable to climate change.

The fact that winter temperature did not affect Black-capped chickadees’ problem solving abilities and responses to novelty suggests that annual variation in winter temperature will not have an effect on wild populations. It is worth noting, however, that I examined differences after only one year of warming, and it is possible that longer term warming does influence behaviour. This is important as climate is expected to continue to change at its current rapid rate. Eventually, as a result of climate change, the chickadee habitat will begin to change. This change in habitat could lead to an increased number of novel feeding situations, where enhanced problem solving abilities could be beneficial. In this case, some flexibility in behavioural responses would be important as it may allow individuals to more quickly exploit these novel food sources. Additionally, these results only directly examined behavioural responses in Black-capped chickadees. Other species may be more sensitive to annual temperature variation, and respond differently.
Winter temperature also had no effect on hippocampal volume. While seasonal variation in the hippocampus has been found in the past (e.g. Smulders et al., 1995), the cue for hippocampal volume change remains unknown. This thesis suggests that temperature is not a regulatory cue for hippocampal volume change. Understanding the mechanism of hippocampal change might help better understand the function of this change, and depending on the regulatory mechanism, it may help us better understand how animals respond to environmental cues.

Breeding condition was affected by winter temperature, and these findings fall in line with the current research that correlates breeding timing with temperature. In order for breeding timing to be flexible, birds need to be ready to reproduce earlier in warmer springs. I have provided evidence that temperature, in the absence of a changing photoperiod, can affect reproductive condition. This provides evidence of a mechanism that birds may use to modulate reproductive timing to maintain fitness. The implications of this are most interesting in a global climate context. As temperatures continue to rise, the reproductive season will likely continue to get earlier, moving progressively further from historical dates and by extension, further from the typical photoperiodic cycle. The ability to use temperature to track optimal lay date then, will become increasingly important.

5.3 Future directions and next steps

While this research has provided some answers, it has also opened the door for a broad range of future research. Tests of behaviour and cognition provided evidence of a treatment by year interaction that warrants further investigation. Tests of hippocampal volume revealed no significant differences between treatments, however, birds that received additional heat did appear to have slightly greater hippocampal volume than control birds. This may be indicative of a missed effect due to sampling date, and thus further studies should be
completed. It is also worth noting that seasonal changes in hippocampal neurogenesis have been observed in Black-capped chickadees (Barnea & Nottebohm, 1994; Smulders et al., 1995; Hoshuoley & Sherry, 2004; Hoshuoley et al., 2005; Hoshuoley & Sherry, 2007). Quantifying hippocampal neurogenesis in temperature treated birds is a logical next step as well. Tests of reproductive condition revealed more developed gonads in birds that received additional heat, however, the sample size for this project was small. Future studies could seek provide more evidence on whether temperature has a direct effect on reproductive condition.

This research provides a basis for further investigation into the effects of overwinter temperature in general. Overwinter temperature can be investigated for its effects on other aspects of avian behaviour and cognition. For example, caching behaviour is assumed to be essential for overwinter survival in some bird species, and the harsher the environment, the more an individual should then rely on caches and spatial memory. The effects of winter temperature on caching decisions, the kind of food cached, the timing of cache recovery, and many other questions could readily be investigated.

5.4 General Conclusions
Temperature can have a range of effects on a wide variety of taxa, and these effects can have varying ecological impacts. For small, non-migratory birds like Black-capped chickadees, these effects can be particularly evident in winter. The purpose of this thesis was to investigate the effects of overwinter temperature in a population of Black-capped chickadees likely to be quite genetically homogeneous and in this thesis, I examined these effects in three distinct ways: 1) the effect of temperature on behaviour and cognition; 2) the effect of temperature on hippocampal volume; and 3) the effect of temperature on reproductive timing. I found that temperature affects reproductive condition, but not behaviour and cognition or hippocampal volume.
These results should prompt future research into the effects of temperature and other environmental variables, and their effects on avian populations. As the threat of climate change continues, understanding how changing environments will impact not just birds, but also animal populations in general, is increasingly important. In order to inform present and future conservation and policy decisions, we need to better understand how populations are responding to change. This includes further investigation into how animals respond, in the short term, to changing environmental conditions.
5.5 References


springs lead to mistimed reproduction in great tits (Parus major). Proceedings of the Royal Society of London B: Biological Sciences, 265, 1867-1870.
Appendix I – Additional Figures: Chapter 2

Figure A1.1 Males and females did not differ in fat mass at any of the three measurement times. Measurements were taken before, during, and after the heating treatments were applied.
There was a statistical difference between year one and two only during Post-Treatment: year two birds had greater fat mass. This is likely due to differences in sampling time. Year one Post-Treatment measurements were taken in May, while year two measurements were taken in April.
Figure A1.3 Male Black-capped chickadees had significantly more lean mass than females at all three measurement points. Measurements were taken before, during and after the heating treatments were applied.
Figure A1.4 Birds from year one were larger than birds from year two in both the Pre-Treatment, and Post-Treatment time points. There is no significant difference at During-Treatment. This is likely because males have a higher lean mass than females, and the M:F ratio in each year is not the same. Year one had a higher M:F ratio than year two.
Figure A1.5 There were no sex differences in problem solving ability. The red horizontal line represents the maximum trial time, with each sex containing multiple individuals scoring as “did not complete”.
Figure A1.6 There were no differences in problem solving abilities between birds from year one, and birds from year two. The red horizontal line represents the maximum trial time, with each year containing multiple individuals scoring as “did not complete”.
Figure A1.7 There are no sex differences between male and female problem solving ability. While males showed less variation in latency, there were individuals of both sexes that completed the task almost immediately, and individuals of both sexes that did not complete the task.
Figure A1.8 Both males and females responded similarly to the novel food dish. Males showed less variation, however, this variation falls well within the variation shown by females.
Figure A1.9 Birds from year one and year two responded similarly to the novel food dish.

There were no statistical differences between years.
Appendix II – Permissions Note; Birds of North America

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August 9, 2017
Jeff Martin
MSc Candidate, Sherry Lab
Advanced Facility for Avian Research
Western University
London, ON, N6G 1G9
Canada

Dear Jeff:

The Cornell Lab of Ornithology appreciates your request for permission to use a range map from the Birds of North America within your Master’s Thesis. As copyright co-holders with the American Ornithological Society, the Cornell Lab of Ornithology will be pleased to grant you permission to utilize the Black-capped Chickadee range map in your thesis.

The following conditions apply:

- This permission and use is not transferable to any other product or publication. Any use of the map image other than as herein described will again require the permission of the Cornell Lab of Ornithology.
- The Cornell Lab of Ornithology gives no exclusive rights to any author, publisher or organization.
- It is understood that you may not use or authorize others to use this information for any purpose whatsoever, other than as herein indicated.
- We would request an acknowledgement something along the order of “from Birds of North America https://birdsna.org, maintained by the Cornell Lab of Ornithology”

Please contact me if you have any questions about the above conditions, otherwise, please sign below.

The foregoing has been agreed to and accepted by authorized representatives from each organization whose signatures appear below:

Jeff Martin

Cornell Lab of Ornithology

Date

Barry Bermudez

Date

Our mission: To interpret and conserve the earth’s biological diversity through research, education, and citizen science focused on birds.
Appendix III – UWO Animal Use Protocol Approval

2015-019:1:

**AUP Number:** 2015-019  
**AUP Title:** Cognition, behavior, and the brain of birds.  
**Yearly Renewal Date:** 11/01/2016

The *YEARLY RENEWAL* to Animal Use Protocol (AUP) 2015-019 has been approved, and will be approved for one year following the above review date.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

**REQUIREMENTS/COMMENTS**  
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Schoeller, Marianne  
on behalf of the Animal Use Subcommittee

*The University of Western Ontario*  
Animal Use Subcommittee / University Council on Animal Care  
Health Sciences Centre, ♦ London, Ontario ♦ CANADA – N6A 5C1  
PH: 519-661-2111 ext. 86768 ♦ FL 519-661-2028  
Email: auspc@uwo.ca ♦ [http://www.uwo.ca/animal/website/](http://www.uwo.ca/animal/website/)
Appendix IV – Canadian Wildlife Service Permit

CANADIAN WILDLIFE SERVICE - PERMIT
PERMIS - SERVICE CANADIEN DE LA FAUNE

Organization
University of Western Ontario

Name
David

Date of issue
November 07, 2016

Date of expiry
October 20, 2019

Special Conditions - Conditions spéciales
1. Prior to any use of this permit, local game authorities (OMNR) are to be notiﬁed relative to collecting procedures, times and localities of collection.
2. Landowner’s permission must be obtained prior to collecting on private property.
3. Permit or a copy of the permit to be carried in the ﬁeld by all collectors.
4. The permit holder is authorized, for scientiﬁc research purposes, to live capture and to hold in captivity a maximum of 50 adult Black-capped Chickadee (Parus atricapillus) per year up to a maximum of 96 adult Black-capped Chickadee over the permit’s validit from sites as located in Southwestern Ontario.
5. A maximum of 25 adult Black-capped Chickadees may be sacriﬁced per year of the permit’s validity for further analysis.
6. Specimens are to be transferred to the University of Western Ontario (London) for further study.
7. Any specimens not retained for study purposes to be disposed of by burial or by approved laboratory waste disposal methods.
8. All specimens that are to be released must be done so at the site of their initial capture.
9. All collected birds to be humanely handled, sampled, and released according to Animal Care Committee protocol of the University of Western Ontario.
10. Any changes to the Animal Care Committee protocol during the annual review process must be submitted to Environment and Climate Change Canada for review and will result in a re-approval process for the permit.
11. Nestbox or young birds in nests are not to be disturbed, damaged, or destroyed.
12. Permit holder shall submit a written report, by 31 January of 2017, 2018, 2019 and 2020, indicating the results of the study to the Canadian Wildlife Service, 867 Lakeshore Road, Burlington, ON, L7S 1A1.
13. Nominees authorized to act under the direction of the permittee are: Seasonal assistants as acting under the immediate direction of the permit holder.
CURRICULUM VITAE

Robert Jeffrey Martin

July 2017

Department of Biology
Advanced Facility for Avian Research
University of Western Ontario
London, Ontario, Canada
N6A 5C2

Academic History

<table>
<thead>
<tr>
<th>Degree</th>
<th>Year</th>
<th>Field</th>
<th>Institution</th>
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<tr>
<td>PhD</td>
<td>In Progress</td>
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<td>University of Western Ontario</td>
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<tr>
<td>MSc</td>
<td>Exp. 2017</td>
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<td>University of Western Ontario</td>
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<td>Northwestern Oklahoma State Univ.</td>
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<tr>
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<td>2015</td>
<td>Health and Sports Science</td>
<td>Northwestern Oklahoma State Univ.</td>
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Research Interests

Temperature and cognition in black-capped chickadees (*Poecile atricapillus*)
Research for my Master’s degree examines the effects of seasonal winter temperature on cognition and behaviour in black-capped chickadees.

Temperature and adaptive nest building in zebra finches (*Taeniopygia guttata*)
This collaborative project with researchers at Western University and the University of St. Andrews, Scotland, explores the effects of ambient temperature on nest structure and composition in birds, specifically the ability of birds to adaptively modify their nests to the thermal environment.

Awards and Distinctions

2015 Western Science Entrance Scholarship
University of Western Ontario, London, Ontario, Canada

2015 Academic Honours; Magna Cum Laude
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2015 Outstanding Senior Award
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2015 Capital One Academic All-America Team Member
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2015 Division II Athletics Directors Association Academic Achievement Award
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2015-2011 Undergraduate Academic Scholarship
Northwestern Oklahoma State University, Alva, Oklahoma, USA
2015-2011  Academic Honour Roll  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2014  Division II Athletics Directors Association Academic Achievement Award  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2013  First Place Poster, All Disciplines, Ranger Research Day;  
*Arsenic Testing in Northwestern Oklahoma State University’s Taxidermy Collections and Natural History Museum*  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2013  Selected to represent NWOSU at Research Day at the Capital;  
*Arsenic Testing in Northwestern Oklahoma State University’s Taxidermy Collections and Natural History Museum*  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

**Invited Talks and Presentations**

2017  Invited Speaker, Scientific Research Society  
University of Western Ontario, London, Ontario, Canada

2017  Guest Lecture, Department of Psychology  
University of Western Ontario, London, Ontario, Canada

2016  Guest Lecture, Department of Psychology  
Brescia University College, London, Ontario, Canada

**Teaching Experience**

2017-2015  Teaching Assistant, Department of Biology  
University of Western Ontario, London, Ontario, Canada

**Supervision and Mentorship**

2017  Undergraduate Honours Project Mentor, Department of Psychology  
University of Western Ontario, London, Ontario, Canada

**Professional, Outreach, and Volunteer Activities**

2018-2017  Graduate Student Member at Large, Student Services Committee  
University of Western Ontario, London, Ontario, Canada

2018-2017  Team Leader-Media, Ontario Ecology, Ethology and Evolution Colloquium  
University of Western Ontario, London, Ontario, Canada

2017  Chairperson, 8th Annual Biology Graduate Research Forum  
University of Western Ontario, London, Ontario, Canada
2017 Thesis Presentation Evaluator, BIO4999E Thesis Presentation Day
University of Western Ontario, London, Ontario, Canada

2017 Oral Presentation Judge, Western Student Research Conference
University of Western Ontario, London, Ontario, Canada

2017 Seminar Leader, Western Student Research Conference Presenter Workshop
University of Western Ontario, London, Ontario, Canada

2017 Abstract Evaluation Team, Western Student Research Conference
University of Western Ontario, London, Ontario, Canada

London District Baseball Association, London, Ontario, Canada

2017-2016 Community Science Outreach Visit Series (5 visits), Lawrie Hawkins PS
Let’s Talk Science – University of Western Ontario

2016 Visiting Speaker Student Host; Susan Healy, University of St. Andrews
University of Western Ontario, London, Ontario, Canada

2016 Oral Presentation Judge, Western Student Research Conference
University of Western Ontario, London, Ontario, Canada

2016 Panelist, First Year Biology Exam Preparation Panel
University of Western Ontario, London, Ontario, Canada

2016 Programming, Biology Graduate Research Forum Organizing Committee
University of Western Ontario, London, Ontario, Canada

2016 Community Science Outreach Visit, Lawrie Hawkins Public School
Let’s Talk Science – University of Western Ontario

2016 Assistant Coach; Head Offensive Coach, London Badgers 18U
London District Baseball Association, London, Ontario, Canada

2016-2015 Community Science Outreach Visit Series (5 visits), Lawrie Hawkins PS
Let’s Talk Science – University of Western Ontario

2015 Assistant Coach, London Badgers 18U
London District Baseball Association, London, Ontario, Canada

2015 Special Projects Chair, Student-Athlete Advisory Committee
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2015 Voting Member (Baseball), Student-Athlete Advisory Committee
Northwestern Oklahoma State University, Alva, Oklahoma, USA
2015  President, Conserving Our Ranger Environment (CORE)  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2014  President, Conserving Our Ranger Environment (CORE)  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2014  Seminar Coordinator, CORE Speaker Series - Peter Galvin  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2014  Seminar Coordinator, CORE Speaker Series – Julia ‘Butterfly’ Hill  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2013  President, Conserving Our Ranger Environment (CORE)  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

2013  Treasurer, Biology Student Organization  
Northwestern Oklahoma State University, Alva, Oklahoma, USA

Professional and Academic Affiliations

2017  Student Member  
Animal Behaviour Society

2017  Student Member  
Canadian Society for Ecology and Evolution

2017-2016  Student Member  
Comparative Cognition Society

Conference Presentations


Ben, S., Fewins, M., Frech, B., Hoffman, D., Jones, A., Martin, R.J., Ortiz, J., Quammen, S., Richmond, C., Smith, S. (2014) “Fuzzy occupancy: A modern day look at rodent gullibility.” Ranger Research Day, Northwestern Oklahoma State University, Alva, Oklahoma, USA. [Poster; Authors Alphabetized; Presenting Author]

Martin, R.J., Reinart, M., Conneywerdy, J. (2013) “Arsenic testing in Northwestern Oklahoma State University’s taxidermy collections and natural history museum.” Ranger Research Day, Northwestern Oklahoma State University, Alva, Oklahoma, USA. [Poster]