THE EFFECTS OF HIGH TEMPERATURES ON MATING COMMUNICATION IN SONGBIRDS

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ABSTRACT

Due to climate change, animals around the globe are experiencing the negative impacts of hot temperature extremes. Hot temperatures can affect animals is by inducing behavioral changes. Some of these behavioral changes include reductions in important, fitness-related behaviors such as foraging and mating. One aspect of mating behaviors that high temperatures can impact is communication. In many systems, mating communication consists of a male signaler transmitting a signal to a female receiver, who then uses the signal to assess the male's quality as a potential mate. High temperatures can impact this process at the level of the signaler, the receiver, and the signal itself. In my dissertation, I focus on the effects of high temperatures on mating behaviors in songbirds. Songbirds are a fitting organism in which to test the effects of high temperatures because they have a well-studied communication system, are particularly vulnerable to high temperatures, and are already experiencing population declines due to climate change. In Chapter 1, I assess the impacts of high temperatures on the quantity and quality of signals produced by male signalers. In chapter 2, I assess the effects of high temperatures on the discriminatory ability of female receivers. In Chapter 3, I assess the effects of high temperatures on the salience of the signal itself. I discuss my results in the contexts of research practices and population persistence in the face of rising temperatures.

TABLE OF CONTENTS

Introduction……1

Chapter 1: High temperatures impact the production of sexual signals in a songbird…….....14

Chapter 2: Elevated temperatures reduce discrimination between conspecific and heterospecific sexual signals …….....34

Chapter 3: The effects of high temperatures on the salience of sexual signals in a songbird…...…….…53

Conclusion…….…….…….73

References……...……..…79

Vita……..……..……94

LIST OF FIGURES

Figure I-1. Dissertation summary……..…….....3

Figure 1-1. Song bouts produced during 35°C and 43°C temperature treatments……..…26

Figure 1-2. Syllable duration at 35°C and 43°C air temperature……..……..27

Figure 2-1. Spectrograms of exemplar song stimuli……..……41

Figure 2-2. Percentage of conspecific and heterospecific song stimuli elicited……...……..45

Figure 2-3. Preference function as a measure of discrimination between conspecific and heterospecific songs at 23°C and hot 41°C treatments……...……..46

Figure 2-4. Sampling effort between 23°C and 41° trials…….…….47

Figure 2-A1. Confirmation of preference for conspecific song…….52

Figure 3-1. Female preference for each stimulus pair……63

Figure 3-2. Relationship between strength of female preference and change in syllable duration of male song (35°C-43°C)…….……65

Figure 3-3 - Figure 3. Linear regression of the relationship between male mass and Δ syllable duration (35°C-43°C)……67

INTRODUCTION

CONCEPTUAL FRAMEWORK

Climate change is causing longer, hotter, and more frequent heatwaves (Dosio, Mentaschi, Fischer, & Wyser, 2018), causing more animals to experience the impacts of ecologically unprecedented high temperatures (Stillman, 2019). Heatwaves are relatively short periods of higher-than-normal temperatures (days or weeks vs. months or years) (Meehl & Tebaldi, 2004). These shorter periods can be more dangerous than longer periods of heat because animals are less able to acclimate (C. E. Cooper, Hurley, & Griffith, 2020). One particularly troubling example of the risks high temperatures pose to animals is the mass mortality event. Since the beginning of the 21st century, mass mortality events have been recorded in birds (McKechnie & Wolf, 2010), small mammals (Welbergen, Klose, Markus, & Eby, 2008) , and humans (Zhao et al., 2018). However, heatwaves need not be lethal in order for animals to experience a fitness cost. High temperatures can cause physiological and behavioral declines that lead to loss of fitness as well (Cunningham, Kruger, Nxumalo, & Hockey, 2013). Physiological data, such as thermal maxima and water regulation are often used to create predictive models about species persistence in the face of climate change (Humphries, Thomas, & Speakman, 2002). However, the inclusion of behavioral data is far less common even though it has been shown to improve the accuracy of population projection models (Conradie, Woodborne, Cunningham, & Mckechnie, 2019; McKechnie, Hockey, & Wolf, 2012; Sinervo et al., 2010).The lack of inclusion of behavioral data is particularly a problem for endotherms because studies addressing the effects of temperature on endotherm behavior are wildly underrepresented (Huey et al., 2012; Wingfield & Farner, 1976) in the literature when compared to ectotherms (Angilletta, 2009).

Communication is one example of a behavior critical for an individual's fitness that is affected by temperature. Communication is the transmission of information from a signaler to a receiver (W. Searcy & Nowicki, 2009). The receiver then assesses the information contained in the signal and makes a decision (W. Searcy & Nowicki, 2009). Communication can be critical for reproductive success because it is often used in a mating context. It can serve to transmit a "keep out" signal for males defending

territories or as a "check me out" signal for males advertising to potential mates (W. Searcy & Nowicki, 2009). In a mating context, typically a male will advertise to a female using a signal, and the female will interpret the information contained in the signal to determine whether or not to mate with him (Jennions & Petri, 1997). This process can be affected by heat at multiple steps: 1) Temperature can impact signalers. A change in signaler behavior can impact the quality and quantity of sexual signals which could lead to fewer opportunities to mate; 2) Temperature can impact receivers. A change in receiver ability to interpret sexual signals could lead to fewer opportunities to mate or choosing a lower quality mate; 3) Temperature can impact the signal. A change the properties of a signal may change the information being transmitted – leading to a disconnect between signalers and receivers (Candolin, 2019). It is particularly important to study the effects of high temperatures on mating communication because understanding who mates and who mates with whom will be critical for understanding the evolution of populations and population persistence in the face of climate change (Candolin, 2019).

In my dissertation, I address the effects of high temperatures on communication at age stage of the communication process in songbirds (**Figure I-1**). Songbirds are especially vulnerable to the effects of high temperatures, and many are at risk of extinction heatwaves continue to increase. Therefore, it is critical to understand how temperature affects mating communication as it is critical for their reproductive success and persistence. **In Chapter 1, I address how high temperatures affect singing behavior in male songbirds. In Chapter 2, I address how high temperatures affect discrimination between sexual signals by female songbirds. In Chapter 3, I address how high temperatures impact the salience of sexual signals in a songbird mating system.**

BACKGROUND

Endotherm thermoregulation

Body temperature determines an organism's ability to perform vital physiological functions. Organisms use different strategies to maintain body temperature (Angiletta, Cooper, Schuler, & Boyles, 2010). For example, the thermal environment determines the body temperature of ectotherms whereas

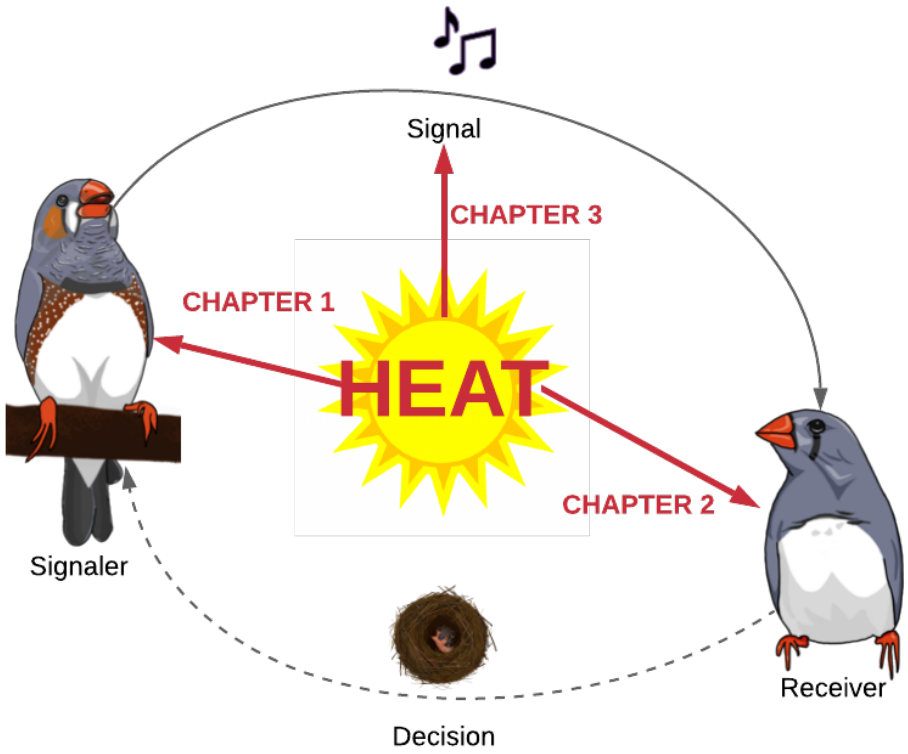

Figure I-1: Dissertation summary. My dissertation addresses the effects of heat on mating communication in songbirds. In Chapter 1, I will focus on how high temperatures impact signalers. In Chapter 2 I will focus on how high temperatures impact receivers. In Chapter 3 I will focus on how high temperature impact the signal itself. A change in any one of these steps could have implications for reproductive success in signalers and receivers.

endotherms maintain body temperature across different thermal environments.

Thermoregulatory processes are conserved across endotherms (Angiletta et al., 2010). All endotherms are best able to maintain their optimal body temperature when ambient temperatures fall within the thermal neutral zone (Angiletta et al., 2010). The thermalneutral zone is the range of ambient temperatures at which an organism has to expend the least amount of energy to physiologically maintain its body temperature; the maximum temperature in this range is called the upper critical temperature, and the minimum is called the lower critical temperature (Angiletta et al., 2010). Temperatures that fall below or above this range induce thermal stress (hypothermia or hyperthermia, respectively) and organisms often need to make behavioral adjustments in response (Angiletta et al., 2010). At a certain point outside of the thermal neutral zone, an organism can no longer physiologically or behaviorally maintain its body temperature, and internal temperature rises or falls (Speakman & Król, 2010). Cold temperatures can decrease thermodynamic energy, causing enzymes to not catalyze reactions at an optimal rate. High temperatures can cause enzymes and other proteins to denature, preventing important chemical reactions that take place within cells, and these changes in chemical processes within the organism can be fatal (Daniel et al., 2010).

Why songbirds?

Small songbirds are especially vulnerable to rising temperatures due to climate change (McKechnie & Wolf, 2010). One reason for this is that they are diurnal, meaning that they are active during the day when temperatures are hottest (McKechnie & Wolf, 2010). Birds also have metabolic rates which cause high temperatures to be more challenging in 3 ways: 1) Birds must combat metabolic heat production, and this heat can be difficult to dissipate at high air temperatures (Hudson, Isaac, & Reuman, 2013). 2) Birds have high water needs, which can be challenging because birds must actively balance water loss with thermoregulation (Dawson, 1982; Speakman & Król, 2010). Additionally, heatwaves are often associated with arid conditions so there is less water available (Albright et al., 2017). Finally, small songbirds have high energy needs- meaning that they need to forage consistently (McKechnie & Wolf,

2004). Foraging is an active process which will produce more metabolic heat, and birds cannot wait until cooler temperatures to forage in order to meet their energy needs (du Plessis, Martin, Hockey, Cunningham, & Ridley, 2012). High temperature are particularly challenging for animals with greater body mass because they have higher metabolic rates and higher energy needs than their smaller counterparts (Speakman & Król, 2010).

Despite facing thermoregulatory challenges, birds exhibit many strategies to cope with higher temperatures. Birds can reduce extra heat production (Christine Elizabeth Cooper, Hurley, Deviche, & Griffith, 2020; Wojciechowski, Kowalczewska, Colominas-Ciuró, & Jefimow, 2020)
Birds canl reduce evaporative water loss and shift their thermalneutral zones (Christine Elizabeth Cooper et al., 2020). Birds will also produce heat shock proteins in response to heat stress (Buchanan, 2000), which help to mitigate the effects of high temperatures at the cellular level (Morimoto, 1996). When physiological shifts aren't enough, birds will also behavioral thermoregulation such as gular fluttering (Nilsson, Molokwu, & Olsson, 2016), panting, tall posture, and wing spreading (Zann, 1996). Birds will also reduce their overall activity (Austin, 1978) and make use of different microclimates (Wolf, Wooden, & Walsberg, 1996). However, these behavioral modifications must balance physiological costs such as water loss (Albright et al., 2017) and meeting energetic needs through foraging (du Plessis et al., 2012). Additionally, many of these adaptations require acclimation to high temperatures. Because heatwaves are relatively short and infrequent (compared, say, to a steady rise average temperature), many birds may not have the opportunity to acclimate – making acute periods of high temperatures more dangerous (C. E. Cooper et al., 2020).

Despite a number of adaptations to climate change, there is mounting evidence that songbirds are facing population declines and extinctions due to climate change. Due to climate change, it is believed that almost 1/3 of extant bird species have disappeared. In North America alone (Rosenberg et al., 2019), it is predicted that a further 2/3 of birds will disappear due to rising temperatures (Bateman et al., 2020). Additionally, we observe a large amount of individual variation in thermal tolerance among individual birds (Wojciechowski et al., 2020). This could lead to substrate upon which selection can act as rising

temperatures due to climate change become a more dire evolutionary pressure (Candolin, 2019). Because of the threat rising temperatures faced by birds, it is critically important to understand how they respond behaviorally acute periods of high temperature, particularly when it comes to behaviors that are linked to fitness.

The role of bird song in sexual signaling

Bird song is a well-studied acoustic signal, which transmits a variety of information including species identity, mate quality, and territory boundaries from a signaler to a receiver (Catchpole & Slater, 2008). Bird song is species specific, however some variation in song can exist between populations and between individuals within a population. In some species including the zebra finch, singing is energetically costly, as song production requires increased oxygen consumption and motor coordination (Franz & Goller, 2003). Song rate can therefore indicate male quality, in terms of competition or mate choice, to receivers (Hauber, Campbell, & Woolley, 2010).

Song can also convey information about species identity because it is species specific. It is important that receivers are able to distinguish between conspecifics and heterospecifics in order to have an appropriate behavioral response, and song is often used in species recognition in birds. Species recognition is especially important in females, which invest more energy into gamete production, and, in some species, into raising offspring. Mating with a heterospecific can waste both time and energy by participating in an unproductive mating attempt (Mendelson & Shaw, 2012). Another possible outcome of mating with a heterospecific is having hybrid offspring. Hybrid offspring are often less fit than their parents or unable to reproduce at all (Servedio & Noor, 2003).

Female birds use song to discriminate between male conspecifics when choosing a mate. Mate choice can be thought of as a combination of two major elements: choosiness and preference (Jennions & Petri, 1997). Choosiness is the amount of energy invested in sampling potential mates. Preference refers to how a female ranks signal quality. When choosing a mate, a female experiences a tradeoff between the energy she invests in searching for a high-quality mate and selecting the male with the signal which she

ranks the highest. Choosing the best quality male has several benefits for female birds, including access to better resources and more fit offspring. However, environmental factors can influence search costs (choosiness) (Jennions & Petri, 1997) and how a female ranks potential mates (preference) causing a female to choose a lower quality mate than she would under ideal conditions (Jennions & Petri, 1997).

STUDY SYSTEM

I selected zebra finches (*Taeniopygia guttata*) as the focal species because they met a number of criteria critical to addressing the objectives of this study. First, there have been a number of studies on thermoregulation of both domesticated and wild zebra finches (Cade, Tobin, & Gold, 1965; Calder, 1964), providing essential information for parameterizing temperatures used in the experimental studies. Second, zebra finches experience heat waves in the wild so assessing the effects of heat stress on behavior is biologically relevant (McKechnie et al., 2012). Third, zebra finches have been well studied for more than five decades in terms of their communication behaviors in both signalers and receivers; thus, I can make use of this foundation of knowledge in measuring effects of heat stress on communication (Hauber et al., 2010; Riebel, 2009). Fourth, zebra finches thrive in captivity and readily exhibit mating behaviors observed in the wild, making it feasible to draw biologically relevant conclusions from captive studies of their mating behaviors (Riebel, 2009).

Zebra finches are native to Australia and New Zealand and experience heatwaves during the their breeding season (Griffith, Mainwaring, Sorato, & Beckmann, 2016). The zebra finch thermal neutral zone ranges from 29.5°C to 40°C (Calder, 1964) or 42°C (Cade et al., 1965) (see **Figure 1**). Zebra finches begin to show an increase in body temperature around 37°C (Wojciechowski et al., 2020). Prolonged exposure (1 hour or more) to temperatures at or above 46°C can be fatal (Cade et al., 1965; Calder, 1964). However, the thermal neutral zone is not a fixed range, and can shift based on various factors such as activity and acclimation. In fact, zebra finches who are acclimated to high temperatures will have higher thermal maxima than unacclimated individuals (Christine Elizabeth Cooper et al., 2020). When exposed to thermally challenging temperatures, zebra finches will increase their water intake to make up for

evaporative water loss (Cade et al., 1965). They also show thermoregulatory behaviors such as decreased movement, panting, wing spreading, and tall posture (Cade et al., 1965; Zann, 1996).

Exposure to thermally challenging temperatures can have negative physiological and behavioral effects on zebra finches. In some parts of their range, zebra finches experience temperatures greater than 40°C, or the estimated upper critical temperature for more than 50 days out of year (R. M. Danner, Coomes, & Derryberry, 2021). At this temperature, male zebra finches produce a higher proportion of non-motile sperm, possibly reducing their fertility (Hurley, McDiarmid, Friesen, Griffith, & Rowe, 2018). Additionally, at high air temperatures zebra finches have been shown to reduce foraging and social behavior (Funghi, Mccowan, Schuett, & Griffith, 2019), as well as show motor and cognitive declines on foraging tasks (R. M. Danner et al., 2021). According to the IPCC, temperatures are expected to climb further as heat waves become more extreme and last longer (Intergovernmental Panel on Climate Change, 2014). By testing how heat stress affects communication behavior in zebra finches in the lab, we will be able to infer how heat stress may be affecting communication in the wild.

Zebra finches are well-suited experimental subjects for this project because their mating and communication behaviors have been extensively studied in both males and females. In the wild, zebra finches live and breed in large flocks but are socially monogamous (Riebel, 2009). Female zebra finches use both auditory and visual cues when assessing mates. Song and beak color are the two traits that females use to assess quality (Hauber et al., 2010; Riebel, 2009). Females prefer males with redder bills (Burley & Coopersmith, 2010). However, visual cues are not salient when song is not present (Riebel, 2009). Although both sexes vocalize, only males produce the species-typical stereotyped song (Hauber et al., 2010; Riebel, 2009). Male zebra finch song typically falls into one of two categories, directed or undirected (Hauber et al., 2010; Riebel, 2009). Undirected song has no known intended receiver. Directed song is sung to a potential mate (Hauber et al., 2010). Directed songs are produced at a faster rate, contain more introductory notes, and have more motifs per bout than undirected songs (Hauber et al., 2010). Females readily approach directed song (Hauber et al., 2010). Females are the primary receiver, as males are not territorial, and appear

to use song to assess male quality (Hauber et al., 2010; Riebel, 2009). Song preference in female zebra finches is the best predictor of pair formation (Holveck & Riebel, 2007).

The elements of male zebra finch song have been extensively characterized in previous studies (Hauber et al., 2010; Riebel, 2009; Sossinka & Böhner, 1980). Songs are typically comprised of harmonic syllables. These syllables are sung in sequences called motifs. Motifs are then sung in groups called bouts (Hauber et al., 2010). Typically, the male song begins with a few introductory syllables, followed by a variable number of motifs (Riebel, 2009). The number of different syllables within a motif is called repertoire size. Some songs can include special elements, called inspiratory notes, within motifs. Typically, males have "rules" by which their different motifs are arranged called phonetic syntax. However, the same male can utilize different syntaxes (Riebel, 2009). Because song production requires individuals to shift rapidly from silent to sung intervals, singing requires an increase in oxygen consumption and energy use (Franz & Goller, 2003). Therefore, song rate serves as an honest sexual signal to potential mates in this species (Hauber et al., 2010).

The song types and song elements that females prefer have been extensively studied in zebra finches (Hauber et al., 2010; Riebel, 2009). A few components of song preference are heritable, such as species recognition (Riebel, 2009). Females innately prefer songs of their own subspecies (Riebel, 2009). Other components of song preference are learned. For example, zebra finch females typically prefer their tutor's song (Holveck & Riebel, 2007) or other familiar songs (Hauber et al., 2010; Riebel, 2009). Additionally, they show strong preference for the directed song of their own mate (Hauber et al., 2010). Some studies show that female zebra finches prefer males who sing at higher rates, and females who choose males who sing higher rates have sexier sons (Houtman, 1992). Some females also show preference for longer songs (Clayton & Pröve, 1989) and longer motif duration (Neubauer, 1999). .

One potential drawback of using the zebra finch as the focal species is that zebra finches are domesticated. Domesticated populations can diverge from wild populations (Riebel, 2009). Additionally, due to years of breeding, no two populations of domestic zebra finches are genetically the same (Riebel, 2009). Thus, it is necessary to account for this evolutionary history in making biologically relevant

interpretations of the effects of heat stress on mating behaviors. Because both wild and captive populations have been studied, we can account for some of these potential effects. In experiments comparing mate choice behaviors of both domestic and wild-caught zebra finches, researchers have shown that domestic and wild-caught zebra finches show similar behaviors (Rutstein, Brazill-Boast, & Griffith, 2007). In these trials, both types showed similar activity levels and both preferred males of their own type (domesticated or wild-caught) (Rutstein et al., 2007). Further experiments show that life history strategies and investment trade-offs are similar between wild and domestic zebra finches despite facing different selective pressures (Tschirren, Rutstein, Postma, Mariette, & Griffith, 2009). When testing hypotheses regarding life history and sexual selection in captive zebra finches, the explanations are likely still applicable to wild populations (Tschirren et al., 2009). Additionally, general patterns of thermoregulation and thermal sensitivity are conserved across endotherms (Angiletta et al., 2010). All endotherms perform best within a given range of ambient temperatures, and perform poorly beyond that range (Angiletta et al., 2010). Additionally, previous work has found no difference in the thermoregulation of wild-caught and domestic zebra finches (Calder, 1964; Marschall & Prinzinger, 1991). It is highly unlikely that wild and domestic zebra finches have diverged to the point of using different thermoregulatory strategies. Thus, although the effects of domestication will need to be considered, these effects do not prevent us from making biologically relevant interpretations of results from our captive experimental studies.

DISSERTATION CHAPTERS

Chapter 1: High temperatures impact the production of sexual signals in a songbird

In this chapter, I assessed the effects of high temperatures on song production in male zebra finches. I hypothesized that hot temperatures would impact both quantity and quality of male sexual signals, as prior research has shown that songbirds sing less at high temperatures (Luther & Danner, 2016) and that song features, such as syllable duration, are impacted by brain temperature in zebra finches (Aronov & Fee, 2012). I exposed male zebra finches to two different temperature treatments: 35°C and 43°C, placed a female zebra finch within view, and recorded all directed songs produced. I then counted all directed songs

and found that males sing fewer songs at 43°C than at 35°C. I then assessed whether syllable duration was different between the two treatments. I found that songs produced at 43°C had shorter syllables than songs produced at 35°C. I also found that syllables were more likely to be sung in a different order during the 43°C treatment than the 35°C treatment, indicating a change in song structure due to temperature. However, songs produced at 43°C were overall more similar and had higher rates of acoustic match than songs produced at 35°C. This could indicate a decrease in the variability with which zebra finches are able to produce songs at higher temperatures.

Taken together, these results mean that male zebra finches could experience reduced reproductive success when coping with hot temperatures. A reduction in song rate may lead to fewer opportunities to mate, as females prefer males who sing with higher song rates (Houtman, 1992). Additionally, shorter notes lead to shorter motifs. Females often prefer males who sing longer motifs due to increased signal exposure (Clayton & Pröve, 1989; Neubauer, 1999). Consistent with previous work, I also found a large amount of individual variation in change in song production between treatments among males, indicating that some males are likely more thermally sensitive than others. In the wild, this pattern could lead to natural or sexual selection against males who are more thermally sensitive.

Chapter 2: Elevated temperatures reduce discrimination between conspecific and heterospecific sexual signals

In this chapter, I explored the effects of high temperatures on discrimination between sexual signals in female zebra finches. Using an auditory discrimination assay called operant conditioning, I asked female zebra finches to discriminate between conspecific and heterospecific songs at 22°C and 41°C. I predicted that female zebra finches would discriminate between the two song types at 22°C but not at 41°C, and that zebra finches would more strongly prefer conspecific songs in the 22°C treatment compared to the 41°C treatment. I also predicted that female zebra finches would reduce how often they sampled songs (e.g., be less choosy) at 41°C.

Consistent with my predictions, I found that female zebra finches preferred conspecific songs in the 22°C treatment but not at the 41°C treatment. Female zebra finches more strongly preferred conspecific songs at 22°C than at 41°C. Contrary to my prediction, I found no difference in sampling effort between treatments. This pattern could be due to a reduction in cognitive performance in female zebra finches (R. M. Danner et al., 2021), or distraction from the stimuli due to the stressor (Berglund, 1993). Overall, these results indicate that high temperatures may impact how females rank sexual signals in the wild, which could lead to females mating with lesser quality males, which could cause a reduction in fitness.

Chapter 3: The effects of high temperatures on the salience of sexual signals in a songbird

In this chapter, I assess whether high temperatures impact the salience of sexual signals. In Chapter 1, I found that males sing syllables with shorter duration at high temperatures. In theory, this change in song acoustics could lead to changes in the information contained by the song. However, this measure on its own cannot tell us whether the change in the signal will affect a male's ability to communicate with females. Therefore, I asked females to discriminate between songs produced at 35°C and 43°C from males recorded in Chapter 1 using operant conditioning. All song pairs were created using songs from the same male in the two different temperature treatments. All females were presented with two pairs of songs from two different males. Each female heard a different combination of male songs. I had no *a priori* predictions about which songs females would prefer, as I didn't know if females would respond to differences in syllable duration.

I found that some females preferred 35°C songs, while other females preferred 43°C songs. When exploring why females showed differences in preferences for songs produced at different temperatures, I found that females who preferred 35°C songs showed greater strength of preference for 35°C songs that had greater duration than the 43°C song in that pair. Females that preferred 43°C songs showed greater strength of preference when 43°C songs were longer than, or close to the same length as, 35°C songs. I then assessed whether change in duration was correlated with features of male morphology such as mass

and found that larger males showed greater reduction in syllable duration between the 35°C and 43°C treatments. This pattern of results indicates that high temperatures do in fact affect the salience of male sexual signals. However, the degree and direction of change is dependent the thermal sensitivity of individual males and individual preferences of females.

INTELLECTUAL MERIT

This is the first set of studies that addresses the effects of high temperatures on mating behaviors in a songbird. Additionally, these studies address this process from three different perspectives: the signaler, the receiver, and the signal itself. Chapter 1 is the first study to demonstrate that high environmental temperatures can impact the quantity and quality of male song. Chapter 2 is the first study to address how high temperatures impact female preference for sexual signals. Chapter 3 is the first study to demonstrate that temperature can affect the salience of songs. Taken together, these studies add to a growing body of research examine the effects of climate change on endotherm behavior, specifically mate choice, which is critical for reproduction and fitness. In the long term, these results, along with other studies addressing the effects of temperature on endotherm behavior, can help to make predictions about population persistence in the face of rising temperatures.

CHAPTER 1

HIGH TEMPERATURES AFFECT THE PRODUCTION OF SEXUAL SIGNALS IN A SONGBIRD

Disclosure Statement: Casey Coomes designed and implemented the experiments contained in this chapter. This chapter has been submitted as a manuscript to *Animal Behavior,* with coauthor and supervisor Dr. Elizabeth Derryberry. Casey Coomes is the wrote the manuscript with revisions from Dr. Elizabeth Derryberry.

ABSTRACT

Global climate change is causing heatwaves to increase in number, length, and intensity. These extreme temperatures can reduce fitness when mating behaviours are affected. An important mating behaviour for many organisms is communication. For example, songbirds, such as the zebra finch (*Taeniopyggia guttata*), use song to attract mates. Here, we test how an acute period of extreme heat affects song production in male zebra finches. We find that birds significantly reduce song output at temperatures that induce heat dissipation behaviors. We also find that birds produce song bouts with shorter syllables when thermally challenged. Further, the relative consistency of songs (a comparative metric) changes with temperature. Altogether, we demonstrate that heatwave-like conditions can impact communication via alterations in signaler behavior and the signal itself. We also discuss the potential compounding effects of reduced song production and other physiological declines on fitness in free-living zebra finches which increasingly experience these high temperatures.

INTRODUCTION

Global climate change is causing an increase in extreme weather events such as heatwaves (Intergovernmental Panel on Climate Change, 2014). Heatwaves become biologically relevant when ambient temperatures exceed thresholds at which species experience fitness costs (Cunningham, Kruger, et al., 2013). One way animals can experience fitness costs is through reductions in mating behaviour (Andersson, 1994). Despite being important drivers of population persistence, data about changes in mating behaviour are not typically included in predictive models exploring the effects of climate change on animal population trajectories (Huey et al., 2012). It is imperative to include these data because who gets to mate and who mates with whom are important drivers of evolution (Andersson, 1994). In fact, physiology-based extinction projection models that have incorporated information about how high temperatures affect mating behaviours make more accurate predictions about population persistence in the face of climate change and severe temperature fluctuations in ectotherms (Sinervo et al., 2010) and in endotherms (Conradie et al., 2019; McKechnie et al., 2012). A key limiting factor is a relative dearth of

information about how temperature affects mating behaviours in endotherms. Thus, there is a pressing need to measure how extreme but ecologically relevant high temperatures impact mating behaviours in endotherms.

Communication, or the transmission of information from a signaler to a receiver (W. Searcy & Nowicki, 2009), is one example of a mating behaviour that can be affected by extreme high temperatures. Decades of work on ectotherms (regulation of body temperature depends on external sources) demonstrate that signalers vary communication behaviours with temperature. For example, the rate of signal production typically varies positively with temperature in ectotherms. Male frogs call at higher rates in warmer temperatures (Zweifel, 1959), the pulse rate of woodhouse toad calls increases with temperature (Sullivan, 1982), and male crickets increase chirp rate and reduce interchirp intervals when exposed to high temperatures (Ciceran, Murray, & Rowell, 1994). In many cases, receivers shift their communication behaviours in tandem with signalers, thus preserving the function of mating behaviours across temperature regimes. For example, this pattern is found in tree hoppers (Jocson, Smeester, Leith, Macchiano, & Fowler-Finn, 2019), tree frogs (Gerhardt, 1978), and ultrasonic moths (Greenfield & Medlock, 2007). In contrast, much less is known about how high temperatures affect communication in endotherms, in part because the ability to self-regulate body temperature can minimize the effects of extreme temperatures.

Among endotherms, small songbirds, which rely on acoustic communication to reproduce, are particularly vulnerable to extreme temperatures (McKechnie & Wolf, 2010). Bird song has a number of functions in mating contexts, including acting as a 'keep-out' signal to defend breeding resources and as a mate attraction signal (Catchpole & Slater, 2008). Depending on the species, males and females can act both as signalers and receivers of information contained in song (Riebel, Odom, Langmore, & Hall, 2019). Small songbirds are diurnal and are active during the hottest parts of the day (McKechnie & Wolf, 2010). They also have high metabolic rates, meaning that they have to forage actively even during periods of extreme temperatures in order to meet their energy needs, and they have to combat production of metabolic heat in high temperatures (McKechnie & Wolf, 2010). A number of studies on both small and

larger bird species adapted to arid regions (including, SW North America, South Africa, Australia) find that temperatures in these regions are already exceeding thresholds that result in fitness costs (Conradie et al., 2019; Cunningham, Martin, Hojem, & Hockey, 2013; du Plessis et al., 2012; Hurley et al., 2018; Iknayan & Beissinger, 2018). Such fitness costs are predicted to lead to population declines among avian populations (Conradie et al., 2019). As such, if extreme thermal events negatively affect either signalers or receivers, this could in turn reduce mating success.

What little research that has been done suggests temperature affects songbird receivers. For example, female Lincoln's sparrows (*Melospiza lincolnii*) prefer male songs previously heard during cold temperatures over songs they heard under warmer temperature conditions (Beaulieu & Sockman, 2012). Pied flycatcher females (*Ficedula hypoleuca*) spend less time assessing potential male mates in cold temperatures (Slagsvold & Dale, 1994). Female zebra finches reduce discrimination between conspecific and heterospecific sexual signals while at hot temperatures (Coomes, Danner, & Derryberry, 2019). Thus, extreme temperatures, both cold and hot, appear to have a negative effect on female mating preferences, which could lead to fitness costs for female receivers. However, the effects of temperature on songbird signalers are less clear.

Experimental and observational work do not always align regarding how temperature affects songbird signalers. At low temperatures, experimental and observational findings are largely consistent. For example, free-living male zebra finches decrease song rate when overnight temperatures are low (Dunn & Zann, 1996). This is consistent with experimental work on captive male zebra finches showing that song rate decreases when the temperature of brain regions important in song production are lowered (Long & Fee, 2009). However, experimental and observational work are not consistent in regard to the effects of high temperatures on song production. Although higher brain temperature is associated with higher song rates (Long & Fee, 2008) and higher song tempo (Aronov & Fee, 2012), observational work shows that male song sparrows (*M. melodia*) sing less when temperatures are higher (Luther & Danner, 2016). This inconsistency could be due to differences among species in how they respond to high temperatures or to mechanisms other than brain temperature affecting song output at high temps.

Therefore, a critical gap in our understanding of how temperature affects communication in songbirds is how high temperatures affect song production.

In this study, we tested how acute exposure to high temperature affects song production in a species already experiencing the negative effects of heatwaves in its native range: the zebra finch (Griffith, Mainwaring, et al., 2016; McKechnie & Wolf, 2010). The number of heat waves in Australia is increasing (McKechnie et al., 2012), with heatwaves often lasting several days and average ambient day temperatures above 40°C (Griffith, Mainwaring, et al., 2016). Mass mortality events in this species during heatwaves are common (McKechnie & Wolf, 2010). Zebra finch body temperature is typical for passerines (~42°C) but their thermoneutral zone is wide (~29.5°C to 40°C) reflecting adaptation to high temperatures (Calder, 1964). This species also shows significant physiological plasticity such that birds can shift their thermoneutral zone to higher temperatures during heatwaves (Christine Elizabeth Cooper et al., 2020). Although, prior exposure does not change their general physiology (C. E. Cooper et al., 2020), limiting their ability to acclimatize to extreme heat events. Like other animals, zebra finches initially respond to heat by moving to cooler microclimates or making minor postural changes to shed heat through convection (Zann, 1996). As ambient temperature exceeds body temperature, heat loss through convection becomes ineffective and, like other animals, zebra finches pant to shed heat through evaporative water loss (Dawson, 1982). Such heat dissipation behaviors require additional energy expenditure and may interfere with other behaviors (Mitchell et al., 2018) such as song production. We therefore hypothesize that male singing behaviour will vary with air temperature.

To test this hypothesis, we measured singing behaviours in male zebra finches at two different air temperatures (35°C and 43°C) using a repeated measures design. We selected 43°C as our high temperature treatment because temperatures above 40°C in this species are known to pose a physiological challenge (C. E. Cooper et al., 2020; Christine Elizabeth Cooper et al., 2020; Wojciechowski et al., 2020). Our cooler temperature treatment (35°C) reflects a marginal thermoneutral condition as birds of this species can develop hyperthermia at air temperatures above ~36°C (Wojciechowski et al., 2020). We

predicted that males would produce fewer songs during the thermally challenging treatment (43°C) than when held in thermoneutrality (35°C). Because syllable duration decreases with increasing brain temperature in this species (Aronov & Fee, 2012; Long & Fee, 2008), we also assessed the effects of temperature on syllable duration. We predicted that songs produced during the high temperature treatment would have shorter syllable duration than songs produced under the lower temperature treatment. Additionally, we used consistency metrics to compare characteristics of songs such as acoustic accuracy and order of syllables (Tchernichovski, Nottebohm, Elizabeth, Pesaran, & Pratim Mitra, 2000a) at different temperatures. Because male zebra finches sing stereotyped songs (i.e., a given male sings the same song every time), consistency metrics can provide insight into how consistently males sing within temperature treatments. We predicted that zebra finches would produce songs with less consistency during the higher temperature treatment. Altogether, we expected to find effects of an acute thermal challenge on singing behavior and the signal itself.

METHODS

Study Species

Zebra finches are a small songbird native to Australia and Indonesia (Zann, 1996), and are an ecologically relevant species in which to study the effects of high temperatures. Zebra finches regularly experience the deleterious effects of heatwaves during their breeding season in the wild (Griffith, Mainwaring, et al., 2016; Hurley et al., 2018). Namely, at high temperatures zebra finches experience a reduction in sperm quality (Hurley et al., 2018), forage less often and are less social (Funghi et al., 2019), change their egg morphology (Dees, Hoffman, & Wada, 2019), and sing more to their embryos which changes how nestlings behave and grow (Mariette & Buchanan, 2016). Zebra finches also thrive and breed readily in captivity (Riebel, 2009), making them ideal for captive, experimental studies. Additionally, there is evidence that thermal limits are conserved between wild and domesticated

populations (Calder, 1964). These factors make zebra finches a useful model in which to test the effects of temperature on song production.

Researchers have extensively studied zebra finch songs in mating contexts (reviewed in Riebel, 2009), providing a framework for making functional interpretations of temperature effects on song. Males sing two types of songs: directed and undirected. Undirected songs are typically sung in social contexts, while directed songs are sung to attract females. A number of studies demonstrate that female zebra finches prefer males with higher rates of song production (Collins, Hubbard, & Houtman, 1994; ten Cate, Verzijden, & Etman, 2006), songs with longer motifs (Neubauer, 1999), and more consistent frequency modulation (Lauay, Gerlach, Adkins-Regan, & Devoogd, 2004).

The elements of directed song are extensively characterized (Sossinka & Böhner, 1980). Songs are typically comprised of harmonic syllables, which are sung in sequences called motifs. Motifs are then sung in groups called bouts. Typically, a male produces the same motif in these bouts, and the consistency with which these motifs are repeated is known as stereotypy. There are several ways to measure this consistency: similarity, accuracy, and sequential match. Similarity is measured as the overall resemblance between two motifs, accuracy compares the differences in acoustic space used by a song, and sequential match compares the order of syllables between two motifs (Honarmand, Riebel, & Naguib, 2015; Tchernichovski, Nottebohm, Elizabeth, Pesaran, & Pratim Mitra, 2000b).

Animal Care and Housing

We tested 16 adult male zebra finches of unknown age. Birds were obtained from Magnolia Bird Farm in Riverside, California, U.S.A. This is a typical sample size for a repeated measures experimental design with captive birds (R. C. Anderson, 2009; R. Anderson, Peters, & Nowicki, 2014; Coomes et al., 2019; Gentner & Hulse, 2000). We housed birds in individual cages (48.26 x 25.4 x 30.48 cm; 19 x 10 x 12 in) lined with newspaper. Each cage contained two perches, and birds were given ad libitum access to food, water, crushed oyster shells, and cuttlebones. Their diets were supplemented with fresh vegetables (snap peas, green beans, etc.) or hard-boiled eggs with shell once per week. The temperature in the

housing room ranged from a minimum of 23°C at night to a maximum of 27°C during the day. The birds were kept on a 13:11 light-dark cycle, with lights-on at 0800 and lights-off at 2100. Humidity ranged between 45% RH and 65% RH. All procedures were approved by the institution's IACUC (Protocol #2578).

Experimental Design

All stages of this experiment took place in thermal chambers constructed from modified sound attenuation chambers (Industrial Acoustics Co., North Aurora, IL, U.S.A.). Each chamber contained a window at the front, a Shure microphone, and two Logitech web cameras to monitor health. Internal measurements of the chamber were 23 x 16 x 14 in. Chambers had constant airflow into and out of the chamber. Incoming air was heated with a heating element which turned on and off to provide accurate temperature control (±0.3°C) with consistent uniformity (±1°C) across a broad range (22—44°C). Humidity differences were monitored using EasyLog temperature and humidity loggers (Lascar Electronics, Erie, PA, U.S.A.). Each temperature chamber was large enough to fit the bird's standard cage allowing us to not handle birds during the course of the experiment. One week prior to the start of the experiment, we placed birds in their home cage individually into the chambers for 2 hours at housing temperature (27°C) to habituate to light and sound differences. In order to determine the temperature for the high temperature treatment, we performed a pre-experimental trial to determine at what temperature our population showed heat dissipation behaviors. We placed birds in a thermal chamber at 35°C and then raised the temperature over the course of one hour until birds showed signs of behavioural thermoregulation. All birds showed at least one or more heat dissipation behaviour at 43°C (panting, wing-spreading, standing tall (Zann, 1996)), so we selected this temperature for our high temperature behavioural trials.

To test how air temperatures affected song production, we then exposed the male zebra finches to the 35°C and 43°C treatments and presented a female zebra finch to elicit directed songs. For both treatments, humidity generally ranged from 10 to 20% rH, depending on the local weather conditions. At

this range, humidity does not constrain thermoregulation via evaporative cooling (Gerson, Smith, Smit, McKechnie, & Wolf, 2014; van Dyk, Noakes, & McKechnie, 2019). Each bird underwent both temperature treatments, and we alternated the order of treatment to reduce order effects. There were two days between each temperature treatment. Each individual underwent each temperature treatment at the same time each day, to reduce the effects of time of day on overall activity levels. Prior to each treatment, we placed a male in a chamber for 30 minutes at temperature to allow them to habituate. After the habituation period, we placed one of a set of five females drawn from the same colony but housed in separate, same sex cages outside the chamber window, so that males could see but not hear the female. A male saw the same female for both temperature treatments to limit any female-specific effects on song. We then recorded all vocalizations produced over 30 minutes using Sound Analysis Pro (Tchernichovski & Mitra, 2004).

Song Analysis

We assessed three measures of singing behaviour: number of song bouts, variation in syllable duration and overall singing consistency. First, we visualized vocalizations using Audacity (The Audacity Team, 2020), then counted all directed song bouts. Song bouts were counted by three observers, two of whom were blind to treatment. Song counts were consistent among all three observers. In order to be counted as a single bout, a vocalization must have had introductory syllables, at least one motif, and have occurred at least 3 seconds from the end of a previous bout (Sossinka & Böhner, 1980). To measure syllable duration, we selected 10 song bouts, or as many as available, from each bird. We excluded songs of low recording quality (i.e. high levels of background noise, breaks in recording, etc.). Then we input the entire bout into the batch analysis feature in Sound Analysis Pro (Tchernichovski & Mitra, 2004). From this batch analysis, we extracted syllable duration.

We then measured overall singing consistency to test whether songs changed under different temperature conditions using standard metrics (Honarmand et al., 2015). To do this, we cut the second motif from each song bout and compared three similarity scores calculated by Sound Analysis Pro:

similarity, accuracy, and sequential match (Tchernichovski & Mitra, 2004). We did not include songs that only contained one motif or songs that were of low recording quality. The resulting data are comparative measurements of consistency between two songs, for example Song A and Song B are 90% similar, compared to Song A and Song C which are only 60% similar. We only compared songs to other songs that were produced by the same bird. These similarity analyses were conducted by an independent observer blind to treatment. We measured consistency within temperature treatments (35°C vs. 35°C, 43°C vs. 43°C). This comparison provides insight into the relative consistency with which a bird sings at a given temperature.

Statistical Analysis

All statistical analyses were performed in R (R Core Team, 2017). To determine whether temperature affects song production, we compared the number of song bouts produced across temperature treatments. We applied generalized linear mixed models with a Poisson distribution, appropriate for count data, using the package lme4 (Bates, Mächler, Bolker, & Walker, 2015). Models included combinations of a fixed effect of temperature as well as a fixed effect of order. We then performed model selection using Akaike's Information Criterion (AIC_c) using the package AICcmodavg (Mazerolle, 2019). All models contained a random effect of male bird to account for the repeated measures design.

To test if temperature explains variation in syllable duration, we applied a linear mixed model, which included temperature treatment as a fixed effect and bird as a random effect. We square root transformed the response variable (syllable duration) to normalize the distribution of residuals. Similarly, to test if temperature impacts consistency of song production, we applied linear mixed models to the measures of song similarity, accuracy, and sequential match for comparisons across temperature treatments. For similarity, we performed Box Cox transformations using the MASS package (Venables & Ripley, 2002). We did not transform accuracy data, as its residuals were normally distributed. For sequential match, we applied generalized linear mixed models with an inverse Gaussian distribution,

appropriate for left skewed data. All models contained temperature as a fixed effect and bird as a random effect.

RESULTS

Song output is lower at high air temperature

We found that our best fitting model contained a fixed effect of temperature and a random effect of bird (**Table 1-1**). The next best-fitting model (ΔAICc <2) contained a fixed effect of temperature and random effects of treatment order and bird. Bird song production changed with temperature treatment (z=-3.21, n=16, p=0.001). Consistent with our predictions, we found that male zebra finches produced significantly fewer directed songs at higher temperature (N=16; **Figure 1-1**). During the 35°C temperature treatment, birds sang an average of 7 song bouts. During the 43°C temperature treatment, birds sang an average of 4.5 song bouts. We also observed a large amount of individual variation (SD_{bird} = 0.65) likely the result of not all birds showing a decrease in song production at high temperature (**Figure 1-1**).

Syllable duration is shorter at high air temperature

Syllable duration varied significantly between temperature treatments (t=-2.42, n= 14, p=0.016). Consistent with our prediction, syllable duration was shorter in songs produced at higher air temperatures (35°C: 103.70 ± 60.22 ms (mean ± SE), 43°C: 89.11 ± 61.70 ms; **Figure 1-2**). Here, we also observed a large amount of individual variation (SD_{bird}=0.62).

Song consistency varies with air temperature

All three measures of song consistency (similarity, accuracy and sequential match) varied with air temperature. We found significant differences in song similarity between temperature treatments (t = -2.59, n=15, p=0.01). Counter to our predictions though, we found that songs produced during exposure to high temperatures had very similar but significantly higher rates of similarity than songs produced at

Table 1-1. Model selection for song count models using Akaike's Information Criterion for small sample sizes.

Model Effects	K	AICc	Δ AICc	AICc Weight	Log-likelihood
Temperature + (1|Bird)	3	223.32	0.00	0.67	-108.23
Temperature + Order + (1|Bird)	4	224.87	1.54	0.31	-107.69
(1|Bird)	2	231.19	7.87	0.01	-113.39
Order + (1|Bird)	3	232.56	9.23	0.01	-112.29

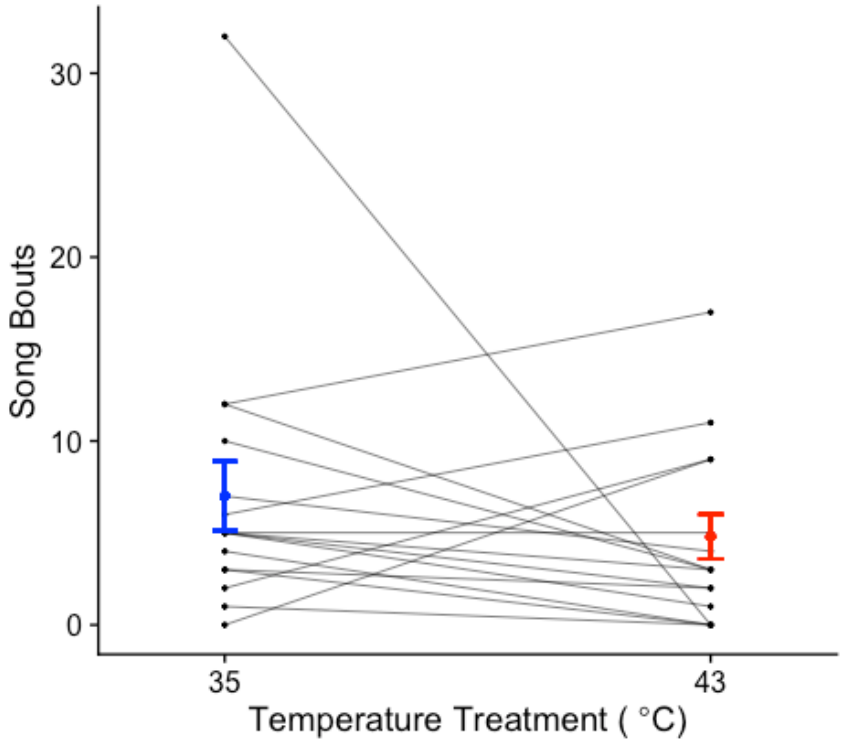

Figure 1-1. Song bouts produced during 35°C and 43°C temperature treatments. Each black line represents an individual bird. The colored lines represent the population-level mean and standard error within each treatment.

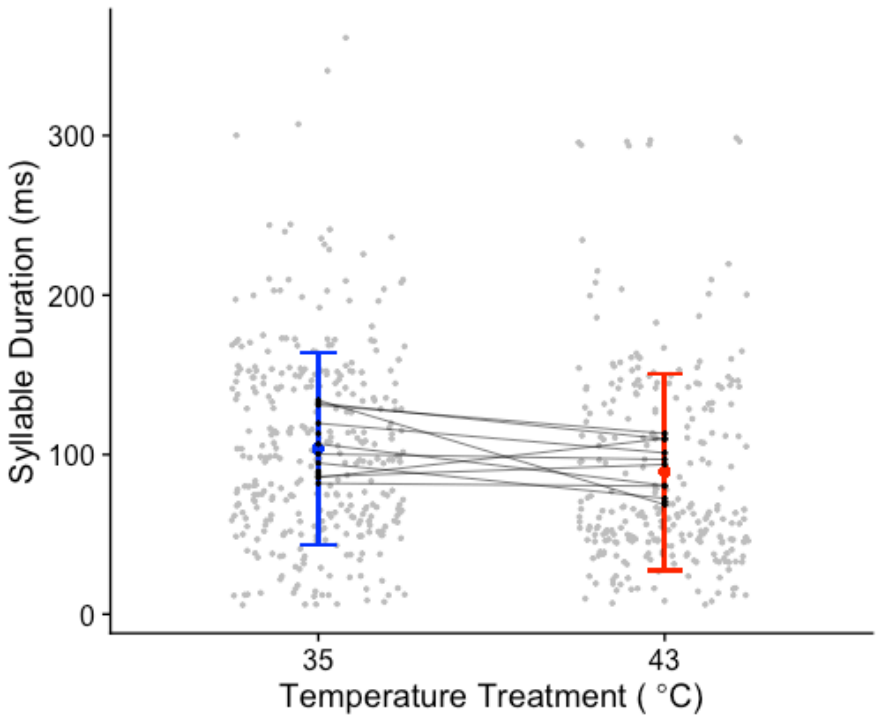

Figure 1-2. Syllable duration at 35°C and 43°C air temperature. Gray dots represent the duration of individual syllables. The black lines represent the change in mean syllable duration for individual birds in each treatment. Colored lines represent the population-level mean and standard deviation of syllable duration.

lower air temperatures (35°C: 89.27± 16.71%; 43°C: 89.84 ± 14.83%). Song accuracy also changed with temperature treatment (t=-4.55, n=15, p<0.0001). Again, songs produced during exposure to higher temperatures also had very similar but significantly higher rates of accuracy (35°C: 84.84 ± 3.87%; 43°C: 85.09 ± 4.76%). Finally, sequential match also varied significantly with temperature treatment (t=2.57, n=15, p=0.01). Consistent with our predictions, we found that songs produced during exposure to higher temperatures had lower rates of sequential match (35°C: 96.61 ± 9.83%; 43°C: 93.81±8.98%). Overall, songs produced at higher temperatures were more similar and more accurate but less consistent in sequential match than songs produced at lower temperatures.

DISCUSSION

Consistent with our predictions, male zebra finches sang fewer song bouts at higher temperatures, indicating an effect of temperature on song production. Also consistent with our predictions, we found that syllable duration was shorter at hotter temperatures. Changes in song consistency varied with temperature. Contrary to our predictions, when assessing changes in song consistency, we found that songs produced at 43°C had higher rates of similarity and accuracy. However, consistent with our prediction, we found that birds had lower rates of sequential match at higher temperatures. Altogether, our results provide strong experimental evidence that exposure to extreme but ecologically relevant high temperatures affects song production in a songbird.

There are several potential reasons why male zebra finches may sing less at higher temperatures. First, it is possible that high temperatures affect males' motivation to sing. In adult male zebra finches, androgen levels are positively correlated with song production (Pröve, 1974). In broiler chickens (*Gallus domesticus*), heat stress has been shown to influence the production of gonadal hormones (Rozenboim, Tako, Gal-Garber, Proudman, & Uni, 2007). While a direct relationship between heat and androgen levels has not been found in male zebra finches, there is some evidence that heat does affect testes, where

androgens are produced, as sperm quality decreases when temperatures reach above 40°C (Hurley et al., 2018). Additionally, pilot data suggest that high temperatures have the potential to impact opioid and dopamine production in the brain in zebra finches (Lipshutz et al In Prep). Dopamine production and opioid release are heavily involved in the motivation to sing and make singing rewarding (Riters, 2010). Another, not mutually exclusive explanation for fewer songs in high temperatures is an overall decrease in social behaviour. Singing is a form of communication, which is inherently social. Broiler chickens have been shown to decrease social interactions at high temperatures (Etches, John, & Gibbins, 2008). Another potential explanation is that males experience tradeoffs in terms of time or energy budgets between song production and heat dissipation behaviours. Birds have already been shown to undergo behavioral tradeoffs between thermoregulation and foraging behavior (du Plessis et al., 2012; Funghi et al., 2019). In zebra finches, time spent singing is energetically costly (Gil & Gahr, 2002). It is possible that male zebra finches are expending more energy thermoregulating and performing heat dissipation behaviours such as panting than they are producing song.

No matter the mechanism, reduced song production could reduce overall reproductive success in male zebra finches. Higher song rates in males elicit more interest from female zebra finches (Hauber et al., 2010), acoustic structure predicts reproductive success in the wild (Woodgate, Mariette, Bennett, Griffith, & Buchanan, 2012), and females who choose males with higher song rates have higher quality offspring and sexier sons (Houtman, 1992). Further, it does not appear to be the absolute song rate but the relative song rate that is important. Males that experience a reduction in song output at higher temperatures will be less effective at attracting mates than males that do not. Therefore, reduced song rates could lead to fitness costs for affected males and differential fitness across individuals, setting up the potential for selection on males during thermal challenges such as heat waves.

Syllable duration also altered under exposure to high temperatures. Reduction in syllable duration during the 43°C treatment may be due to an increase in brain temperature. Previous work has shown that motifs became shorter as brain temperature rose from 38°C to 43° (Aronov & Fee, 2012). Although we

did not measure brain or body temperature in our birds due to the difficulty of taking these data without altering the behavioral output of the birds, previous work on zebra finches did find that male zebra finches at 44ºC experience an increase in body (i.e. cloacal) temperature by ~3.5ºC (Calder, 1964). No matter the mechanisms underlying the decrease in syllable duration, production of shorter syllables may have consequences for mate choice because syllable length is an indicator of age and maturity in male zebra finches; older males sing shorter syllables (Riebel, 2009). The production of shorter syllables leads to production of shorter motifs. It has been suggested that female zebra finches are more likely to prefer males who sing longer motifs because of increased exposure to a sexual stimulus (Clayton & Pröve, 1989). A reduction in motif length may therefore lead to a reduction in mating opportunities.

The differences in consistency with which zebra finches produce songs at different temperature treatments could be explained by temperature's effect on the brain or on motor coordination. Birds varied in their overall song resemblance and use of acoustic space between temperature treatments. Songs produced at 35°C did not match themselves as closely in terms of overall resemblance or acoustic accuracy as did songs produced at the higher temperature, meaning that there was more acoustic variation in the songs produced at 35°C. A potential explanation is that the birds were able to use more acoustic space (there were fewer constraints on song production) when singing at 35°C. These demonstrated changes in song accuracy are consistent with previous studies that show that brain temperature is associated with changes to acoustic properties of song, such as tempo (Aronov & Fee, 2012; Long & Fee, 2008). In contrast, we found that songs produced at 35°C had higher rates of sequential match than songs produced during the 43°C treatment. This indicates that birds more often produced the same notes in the same order at 35°C. One explanation for this pattern is that certain notes may be more difficult to produce under hot temperatures due to motor constraints. Certain notes require more motor skill to produce than others (Goller & Daley, 2001), and male zebra finches have been shown to have reduced motor performance at high temperatures (R. M. Danner et al., 2021).

We also observed individual variation in behavioral performance during this acute thermal challenge. Although many individuals were sensitive to the high temperature treatment, and altered their song performance, some appeared more tolerant. For example, four birds increased song output at high temperature, and two birds showed an increase in syllable duration at high temperature. Individual variation in behavioral performance in heat has also been noted in other studies of the effects of heat on behavior, including female mate choice (Coomes et al., 2019) and foraging behavior (R. M. Danner et al., 2021), suggesting that some individuals are likely more thermally tolerant than others. Thermal tolerance could be generated in a number of ways. For example, some individuals might be more tolerant because they had larger thermal windows (increasing their ability to dissipate heat) (Tattersall, Andrade, & Abe, 2009), more thermal larger mass (produce more metabolic heat) (Speakman & Król, 2010), or differential expression of gene networks related to metabolic rate (Stager, Swanson, & Cheviron, 2015), water balance (McCormick & Bradshaw, 2006), or singing behaviors (Maney, Erwin, & Goode, 2005). The question of why individuals varied in their behavioral performance at high temperatures is beyond the scope of this study. Such empirical questions are critical for future research to address.

Reduced song production and changes in song structure could compound negative effects of heat that zebra finches are already experiencing in the wild. Free-living zebra finches regularly experience temperatures that induce thermoregulatory behaviors (Griffith, Mainwaring, et al., 2016). Researchers have found that male zebra finches have reduced sperm quality when environmental temperatures are above 40°C (Hurley et al., 2018). Zebra finches have also been shown to forage less often during periods of high temperature, reducing overall food intake and social interaction (Funghi et al., 2019). Additionally, researchers have demonstrated that zebra finches produce a unique call that aids thermoregulation when exposed to high temperatures, known as "vocal panting" (Pessato, McKechnie, Buchanan, & Mariette, 2020). Temperature has also been shown to affect how zebra finches rear their young (Mariette & Buchanan, 2016), and may even lead to changes in egg morphology (Dees et al., 2019) and earlier hatch dates (Griffith, Mainwaring, et al., 2016). The additive effects of the physiological and behavioural changes induced during heatwaves could lead to reduced reproductive success for

individuals, and even lead to large-scale consequences such as range shifts and population declines as seen in other species (McKechnie & Wolf, 2010; Sinervo et al., 2010).

As global temperatures continue to rise, it is critical to understand how behavioural and physiological changes induced by extreme temperatures will affect organisms throughout the world. Because zebra finches are a desert adapted species, with relatively low basal metabolic rates compared to other birds and high thermal conductivity (Zann, 1996), they are a conservative organism in which to study the impact of extreme temperature events. In temperate areas, particularly across North America, Europe, and Asia, temperatures are predicted to rise by more than 10°C by 2100 (Intergovernmental Panel on Climate Change, 2014). Temperate zone species have a much lower upper critical temperature, meaning that they will experience deleterious effects of heat at lower temperatures. Additionally, we were unable to find any studies that address the impacts of high temperatures on fitness in temperate songbirds. It is imperative to explore the effects of high temperatures on both tropical and temperate species, in order to predict and potentially mitigate population declines or extinctions due to climate change.

ETHICAL NOTE

All housing protocols and experimental procedures described above were approved by the Institutional Animal Care and Use Committee. Birds were acclimated to the facility for two weeks before beginning any manipulations. We constantly monitored birds while at the 43°C temperature using closed circuit cameras, and immediately removed the bird from the trial if it showed any signs of distress such as gaping or hawk sitting. We also had backup thermal probes inside of each chamber to inform us in case of any unanticipated changes in temperature.

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CHAPTER 2

ELEVATED TEMPERATURES REDUCE DISCRIMINATION BETWEEN CONSPECIFIC AND HETEROSPECIFIC SEXUAL SIGNALS

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ABSTRACT

Heat waves are rapidly increasing in frequency and length around the globe. These periods of elevated temperatures are inducing extreme thermal stress in many organisms. One dramatic result of heat waves is mass mortality events, notably in birds. Although much attention is given to lethal events, little is known about how more common sub-lethal temperatures affect behaviour, especially in endotherms. For example, male song sparrows sing at lower rates at higher temperatures. We hypothesized that simulated heat waves may also reduce the ability of females to discriminate between sexual signals. We tested this hypothesis by quantifying whether female zebra finches (*Taeniopygia guttata*) responded more to conspecific song or heterospecific song while at their typical housing temperature compared to temperatures above their upper critical temperature. We collected data on how often the birds selected conspecific over heterospecific songs and how much effort the birds invested in sampling those songs using a matched pairs design. During housing temperature trials, females preferred conspecific song compared to heterospecific song. In contrast, females no longer showed a preference for conspecific song at temperatures above their upper critical limit, although the birds invested similar effort in sampling songs in both treatments. Our findings provide the first experimental evidence that high temperatures affect female preference for a mating signal in an endotherm. Our results highlight the need for future work to investigate this overlooked effect of climate change on endotherms. Because thermoregulatory processes are phylogenetically conserved, our results generate useful predictions for future studies of heat waves effects on mating behaviours in endotherms.

INTRODUCTION

Climate change is causing an increase in the number, length, and intensity of heat waves worldwide (Intergovernmental Panel on Climate Change, 2014). A heat wave is generally defined as a prolonged period of above average temperature (Meehl & Tebaldi, 2004). Heat waves have drastic effects on animal populations, for example causing mass mortality events in birds (Albright et al., 2017; McKechnie & Wolf, 2010). High temperatures also have sub-lethal consequences by exposing animals to

heat stress, i.e. exposure to temperatures outside of the thermal neutral zone in which an organism can maintain homeostasis (Speakman & Król, 2010). Elevated but sublethal temperatures can impact behaviour, and they can have profound negative consequences on the demography of animals, such as population declines and extinctions (Sinervo et al., 2010).

We know relatively little about sub-lethal effects of high temperatures in endotherms. Most studies focus on physiological changes associated with elevated but sub-lethal temperatures (Cunningham, Martin, et al., 2013; McKechnie & Wolf, 2004; Nilsson et al., 2016; Smit, Harding, Hockey, & E. McKechnie, 2013), but very few have addressed how high temperatures affect animal behaviour (Aublet, Marco, Bergero, & Bassano, 2009; du Plessis et al., 2012). Behavioural changes in endotherms are likely to be most pronounced during heat stress. Heat stress may induce behavioural changes through cognitive declines or changes in motivation (McEwen & Sapolsky, 1995; Riters, 2010; Rozenboim et al., 2007), or through distraction if the animal shifts its attention from the behaviour to an outside stimulus (Mendl, 1999). A few studies have provided evidence that elevated temperatures have the potential to induce behavioural changes in endotherms (Aublet et al., 2009; du Plessis et al., 2012). For example, when temperatures are high, southern pied babblers (*Turdoides bicolor*), consume less food despite spending the same amount of time foraging as when temperatures are lower (du Plessis et al., 2012). However, sufficient data are lacking to make predictions about how endotherms will respond to increasing temperatures due to climate change (Boyles, Seebacher, Smit, & McKechnie, 2011; Huey et al., 2012).

An important behaviour that elevated temperatures have the potential to affect is communication. For example, a recent observational study found that male song sparrows (*Melospiza melodia atlantica*) produce fewer songs when temperatures are high (Luther & Danner, 2016). Songs are vital for communication in birds. For example, they transmit information that can be used to identify and discriminate between conspecifics and heterospecifics (W. A. Searcy & Nowicki, 2005). Species recognition can be thought of as part of mate recognition (Andersson, 1994), and it has been suggested that species recognition and mate choice rely on similar discriminatory mechanisms (Rosenthal & Ryan,

2011). Choice can be thought of as having two major components: preference, or how an individual ranks signals based on their relative quality under similar conditions, and sampling effort, or amount of time or energy an individual puts into assessing signals (Jennions & Petri, 1997). High temperatures have the potential to affect the amount of effort put forth to sample potential mates. For example, female pied flycatchers (*Ficedula hypoleuca*) have been observed to sample fewer males when temperatures are colder, leading to pairings with already mated males (Slagsvold & Dale, 1994). High temperatures could also affect sampling effort is through reduced motivation to mate. In chickens, high temperatures have been shown to suppress gonadal activity and reduce social behaviour (Etches et al., 2008; Rozenboim et al., 2007), which could cause a decrease in motivation to sample sexual signals. There is also potential for elevated temperatures to influence how individuals rank sexual signals. Although some studies have shown that developmental stress does not affect preference (Woodgate, Bennett, Leitner, Catchpole, & Buchanan, 2010; Woodgate et al., 2011), not all stress affects organisms in the same way (Buchanan, 2000). In humans, stress has been shown to induce anhedonia, or inability to have a hedonic response (Pizzagalli, 2015). In birds, opioid production, which is responsible for producing pleasurable responses, has been linked to male motivation to sing (Riters, 2010), and it is possible that these same mechanisms could affect female response to sexual signals. Therefore, heat stress has the potential to make sexual signals less rewarding, thereby affecting signal ranking. Identifying the impacts of heat on communication, and in particular whether those effects are associated with species recognition, is critical in order to clarify the impact of increased temperature on behaviour in endotherms.

Here, we explore the effects of high temperatures on communication by testing the effects of simulated heat waves on species recognition using female zebra finches (*Taeniopygia guttata*). Zebra finches are a useful species to test this question as they have been widely tested in mate preference studies (Hauber et al., 2010; Riebel, 2009). In this species, the male produces a directed song, which the female then uses to identify conspecifics, and confer attractiveness of potential mates (Riebel, 2009). Zebra finches are a biologically relevant system in which to test the effects of elevated temperatures. Because zebra finches thrive in captivity, we can ask questions about how they will respond to elevated

temperatures in a controlled, experimental setting. Past studies have shown that the thermal physiology of wild and domestic populations have not diverged (Calder, 1964). This species is native to Australia and Timor (Riebel, 2009), and populations have experienced mass mortality events due to heat waves (McKechnie et al., 2012). Zebra finches are also likely to experience sub-lethal effects of high temperatures during their breeding season, as temperatures can exceed 40ºC (Griffith, Mainwaring, et al., 2016). The zebra finch thermal neutral zone ranges from 29.5°C to 40°C (Calder, 1964). Above this temperature, zebra finches experience hyperthermia. Zebra finches have been the subject of two recent studies testing the effects of heat stress on fitness. One such study found that male sperm quality decreases during simulated heat waves (Hurley et al., 2018), and the other found that zebra finches change their vocal interactions with their embryos at high temperatures (Mariette & Buchanan, 2016)

We test whether elevated temperatures affect the ability of females to discriminate between conspecific and heterospecific sexual signals, and whether these impacts involve either changes in signal ranking or sampling effort. We predict that zebra finches will reduce their ranking of conspecific song relative to heterospecific songs when experiencing temperatures above their upper critical limit. Additionally, we predict that female zebra finches will sample fewer songs at higher temperatures.

METHODS

Animals and Housing

We tested 13 adult female zebra finches of unknown age. Birds were obtained from Magnolia Bird Farm in Riverside, CA. This is a typical sample size for a repeated measures experimental design testing discrimination using operant conditioning with captive birds (Anderson, 2009; Anderson, Peters, & Nowicki, 2014; Gentner & Hulse, 2000). We housed birds in individual 19 in x 10 in x 12 in (48.26 cm x 25.4 cm x 30.48 cm) cages lined with 2 wooden perches and lined with newspaper, in a room with 32 all female conspecifics. The birds were held on a 13:11 light/dark cycle. Lights on was at 0800 hours and lights off was at 2100 hours. Birds were housed between 22°C and 23°C. We could not control humidity

in the vivarium facility, however the humidity was monitored by the vivarium facility and Institutional Animal Care and Use Committee and generally ranged between 45% rH and 55% rH.

We gave birds *ad libitum* access to Kay-tee Forti-diet finch seed and water, as well as LaFabre's Avi-era vitamin powder supplement, Kayt-ee High Calcium Grit, and cuttlebones. Additionally, birds received fresh food with Kay-tee Molt and Conditioning supplement (boiled eggs, snap peas, or green beans) weekly. All housing and experiments were approved by IACUC (Protocol #0427R).

Operant Conditioning

Overview

We assessed whether female zebra finches discriminate between conspecific and heterospecific songs using an auditory discrimination assay standard for this species: operant conditioning (Hauber et al., 2010). Operant conditioning refers to a learning system in which an organism learns to complete a task through receiving a reward. Here, the reward was the song playback (Holveck & Riebel, 2007). Each operant conditioning unit consisted of the bird's standard cage and a speaker set above the centre of the cage. Two stimulus perches were attached to the front of each cage. These stimulus perches were connected to microswitches, which triggered playback of a song and registered the playback in Sound Analysis Pro (Tchernichovski & Mitra, 2004) using the Live Analysis function. Each perch triggered the playback of the conspecific or heterospecific song stimuli. By choosing which perch to hop on, the bird chooses which song to hear. Because this experiment took place over consecutive days, after each day we alternated the stimulus perches to reduce the effects of side bias. All operant conditioning trials ran from 0800 hours until 1300 hours.

For the conspecific stimuli, we used directed zebra finch songs recorded from prior experiments on males from the same source farm but who were unknown to the subject females. Males were placed in a sound isolation chamber with a female (not a study subject), and all directed songs were recorded. For the heterospecific stimuli, we used rufous-collared sparrow (*Zonotrichia capensis*) songs. These songs were recorded during the pre-breeding and breeding seasons at a time when activity was high, in an open

area to minimize habitat disruptions (Danner, Fleischer, Danner, & Moore, 2017). We selected rufous collared sparrow songs as our heterospecific stimuli as they are structurally very different (**Figure 1**). Zebra finch songs are characterized by repeated individual harmonic notes sung in groups, called motifs (Sossinka & Böhner, 1980). Rufous-collared sparrows sing pure tone songs comprised of whistles and trills (Nottebohm, 1969). All songs were drawn from a pool of 16 conspecific and 16 heterospecific songs (for exemplars, see **Figure 2-1**). All songs were cleaned and volume adjusted to 65dB SPL at the chamber centre. Operant conditioning was split up into three phases: Training, Confirmation of Preference, and Testing. After all birds completed a stage, the next stage immediately began.

Training and Preference Confirmation

For both of these phases, we housed birds in individual sound attenuation chambers (Industrial Acoustics) under normal housing conditions. Training took place over two days. Each day, we used a new pair of song stimuli. We switched the perch that the song stimulus was associated with in order to mitigate the effects of side bias after each day. All birds heard the same song stimuli during training, and we randomized the perches the stimuli were associated with across individuals. In order to pass training, each bird had to hop on both stimulus perches twice, two days in a row. All birds passed operant conditioning training within two days.

Preference Confirmation took place over four consecutive days. We completed this phase to ensure that our population of zebra finches preferred conspecific songs. Each day, the birds heard a new pair of song stimuli. Each bird heard a unique combination of song pairs and we did not reuse songs from the Training phase.

Temperature Trials

We then tested the effects of heat stress on performance of the operant task, using a matched pairs design. Testing took place in operant conditioning units placed inside Conviron Adaptis A1000 model

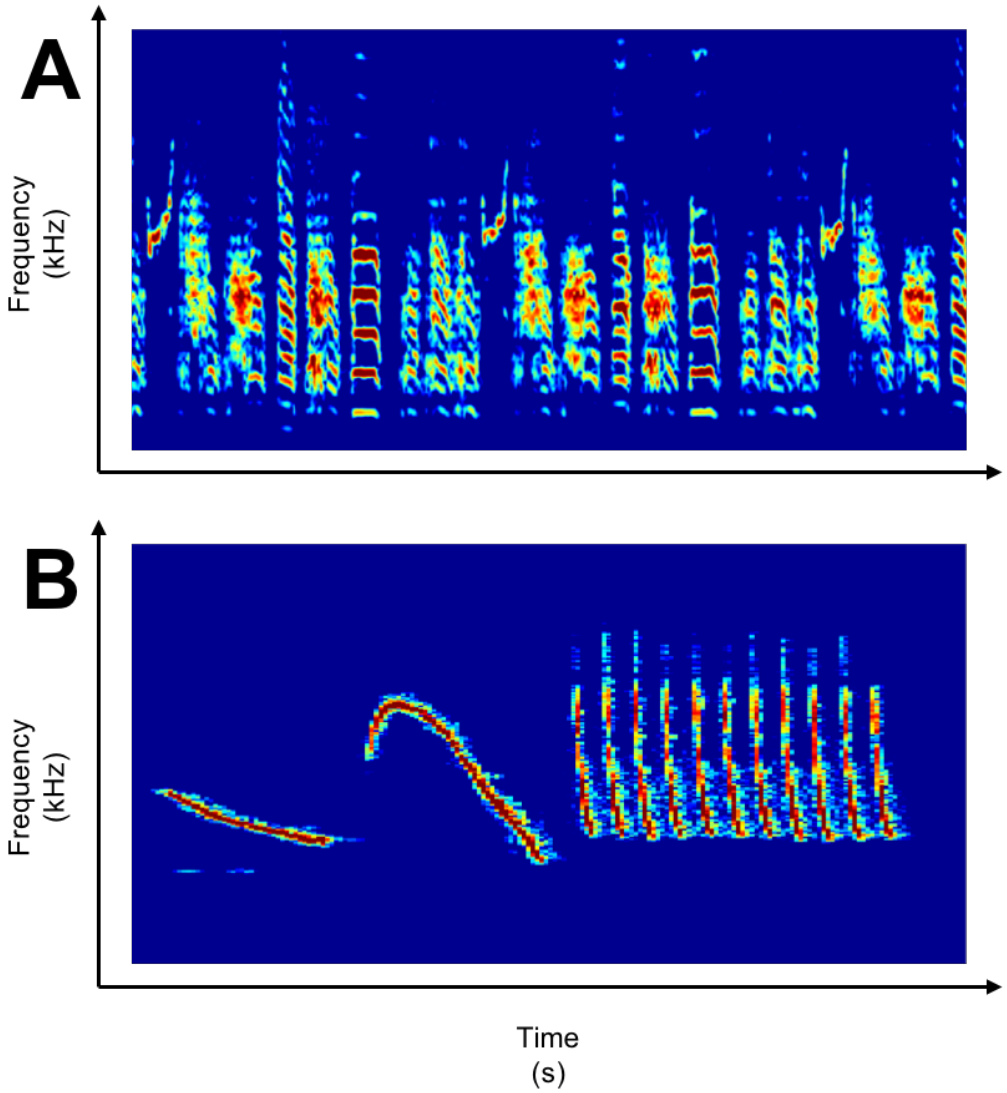

Figure 2-1. Spectrograms of exemplar song stimuli. (A) Conspecific (T. guttata) and (B) heterospecific (Z. capensis) song. Time (2.2 s) and frequency (11kHz) are standardized between the songs.

germination chambers (Controlled Environments Ltd., Winnipeg, Canada), which controlled internal temperature and relative humidity (rH). Birds were allowed to acclimate to the thermal chamber for at least 12 hours before trials began. We used a repeated measures design to control for inter-individual variation, as variation in the level of individual responses to operant conditioning is high (Riebel, 2009). We therefore tested each bird at two different temperatures: 23°C, based on their typical housing temperature in the vivarium (housing temperature) and 41°C (above the zebra finch upper critical temperature). Relative humidity inside of trial chambers generally ranged from 20–30% during hot temperature trials and 50–80% during control trials (similar to the humidity variation in the vivarium facility). The difference between treatments resulted from the fact that relative humidity naturally drops as air temperature increases because warmer air can hold more moisture. In other words, a given amount of moisture in the air leads to lower relative humidity at higher temperatures and higher relative humidity at cooler temperatures. We did not actively control humidity during the trials because the naturally dryer air at higher temperatures ensures little or no compounding effect of humidity on environmental temperature (e.g. "heat index"), whereas higher relative humidity at the lower temperatures has a negligible effect on environmental temperature (Steadman, 1979). Some of the minor variation in humidity was based on the conditions in the surrounding room.

We alternated the order in which individuals underwent the 23°C and 41°C trials to reduce order effects. Each trial took place over four consecutive days, with four days in between the two trials. Additionally, the chamber in which each trial took place was randomized to avoid pseudoreplication due to chamber effects. All birds heard a different combination of stimulus pairs. For a given trial, each subject heard the same pair of songs on days 1 and 2 of the trial, alternating which perch played heterospecific and conspecific song each day, and then a new pair of songs for days 3 and 4 of the trial. Birds did not hear the same two pairs of songs between the two trials. These song pairs were drawn from songs each bird heard during the confirmation of preference phase but presented in novel combinations which ensured that individual birds were familiar with the songs (so any behavioural changes were not

due to unfamiliarity), did not habituate (heard different song pairings), and that individuals heard different sets of stimuli.

Data analysis

Within the preference confirmation phase, 23°C trial, and 41°C and calculated a preference ratio for each individual as the percentage of hops on the conspecific perch out of total perch hops. We then compared this number against a null expectation of 0.5, using Wilcoxon-signed rank tests, a standard statistical approach (Anderson et al., 2014; Holveck & Riebel, 2007). The 0.5 ratio represents the preference ratio if the bird shows no preference for either stimulus. If the conspecific preference ratio is above 0.5, this means that the individual prefers conspecific song over heterospecific song. If it is below 0.5, this means that the bird prefers heterospecific song over conspecific song. Because of the way the preference ratio is calculated (Conspecific Hops/ Total Hops), response to heterospecific song is the remaining ratio, making it the same distance from the 0.5 null as the conspecific ratio. We measured sampling effort as the total number of times a bird hopped on the stimulus perches, as each time they chose to hop on a perch they were sampling a song.

Because each individual underwent both treatments, we then compared the difference in preference ratios between the 23°C and 41°C trials with Wilcoxon signed-rank tests. We also tested whether females showed differences in sampling effort and signal ranking between control and hot treatments. We compared the total number of hops between control and hot treatments (within subjects) using a Wilcoxon signed-rank test. One bird was excluded from all 23°C and 41°C analyses because she reversed her preference for conspecific song between the preference confirmation phase and the 23°C trial. For all analyses, we used Wilcoxon signed-rank tests using the MASS package (Venables & Ripley, 2002) in R (R Core Team, 2017). Our data did not meet the assumptions for parametric statistical tests. In order to calculate effect size (Cohen's d), we extracted the z value using the coin package (Hothorn, Hornik, van de Wiel, & Zeileis, 2006) in R (R Core Team, 2017).

Ethical note

Prior to testing, all birds were acclimated to the facility for at least 3 weeks. All birds were allowed to acclimate to the incubation chambers for a minimum 12 hours before beginning temperature trials. For the first round of high temperature trials, we monitored the birds at 41°C in the presence of a veterinarian. While thermoregulatory behaviours were observed, we observed no signs of distress. After this, the birds were checked every 2 hours during the high temperature trials. Birds were later sacrificed using isoflurane with a secondary method of decapitation, before tissues were harvested for a separate experiment. All work was approved under IACUC protocol 0427-R.

RESULTS

We confirmed that our population of zebra finches preferred conspecific song over heterospecific song during the preference confirmation phase ($X + SE$ = 63.2 + 8.6%, N=13, r=0.86, Wilcoxon signed-rank test: V_{13}=90, P<0.001; **Figure 2-A1)**. Birds ranked conspecific song more highly than heterospecific song during the 23°C treatment ($X + SE$ = 63.4 + 9.7%, N=12, r=0.88, Wilcoxon signed-rank test: V_{12}=78, P<0.001; **Figure 2-2**). As predicted, females did not significantly discriminate between conspecific and heterospecific stimuli during the 41°C treatment ($X + SE$ = 53.4 + 11.2%, N=12, r=0.17, Wilcoxon signed-rank test: V_{12}= 40, P=0.56, **Figure 2-2**).

Nine of the 12 individuals reduced their ranking of conspecific song during 41°C trials as compared to trials, 23°C ($X_{control}+ SE_{control}$ = 63.4 + 9.7%, $X_{hot} + SE_{hot}$ = 53.4+11.2%, N=12, r=0.61, Wilcoxon signed-rank test: V_{12}=56, P=0.034; **Figure 2-3**). Contrary to predictions, we found no difference in sampling effort (i.e., total number of hops) between 23°C and 41°C ($X_{control} + SE_{control}$ =1223.1 + 725.0 hops, $X_{hot} + SE_{hot}$ = 1120.4 + 932.8 hops, N=12, r=0.11, Wilcoxon signed-rank test: V_{12}= 44, P=0.73, **Figure 2-4**).

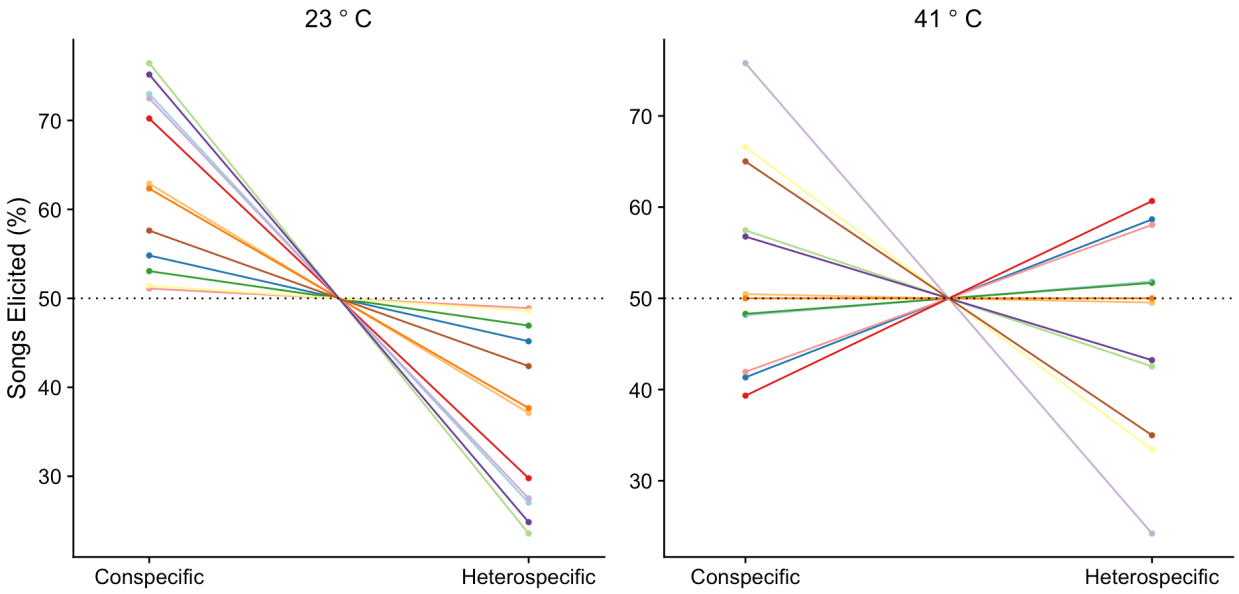

Figure 2-2. Percentage of conspecific and heterospecific song stimuli elicited. Each line represents an individual. Line color denotes the same bird in the 23°C and 41°C trials. The dashed line represents the null (no discrimination between stimuli).

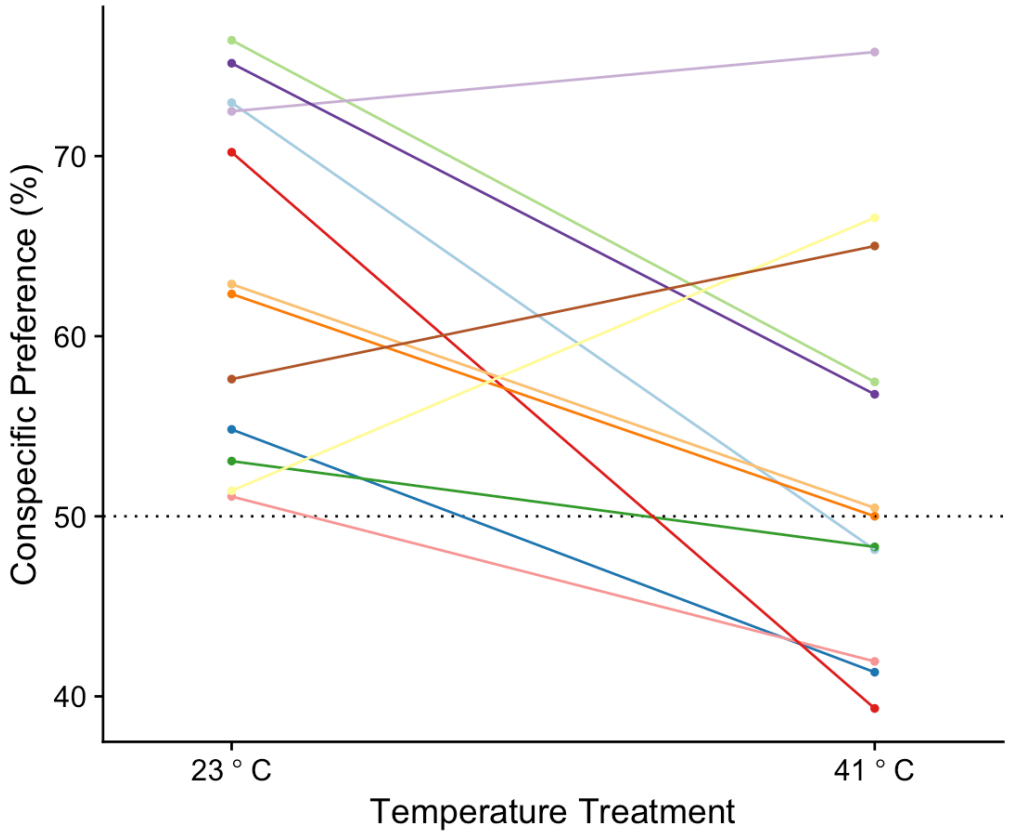

Figure 2-3. Preference function as a measure of discrimination between conspecific and heterospecific songs at 23°C and hot 41°C treatments. Each coloured line represents an individual. A greater percent discrimination indicates a stronger preference for conspecific songs. Dashed line is the null.

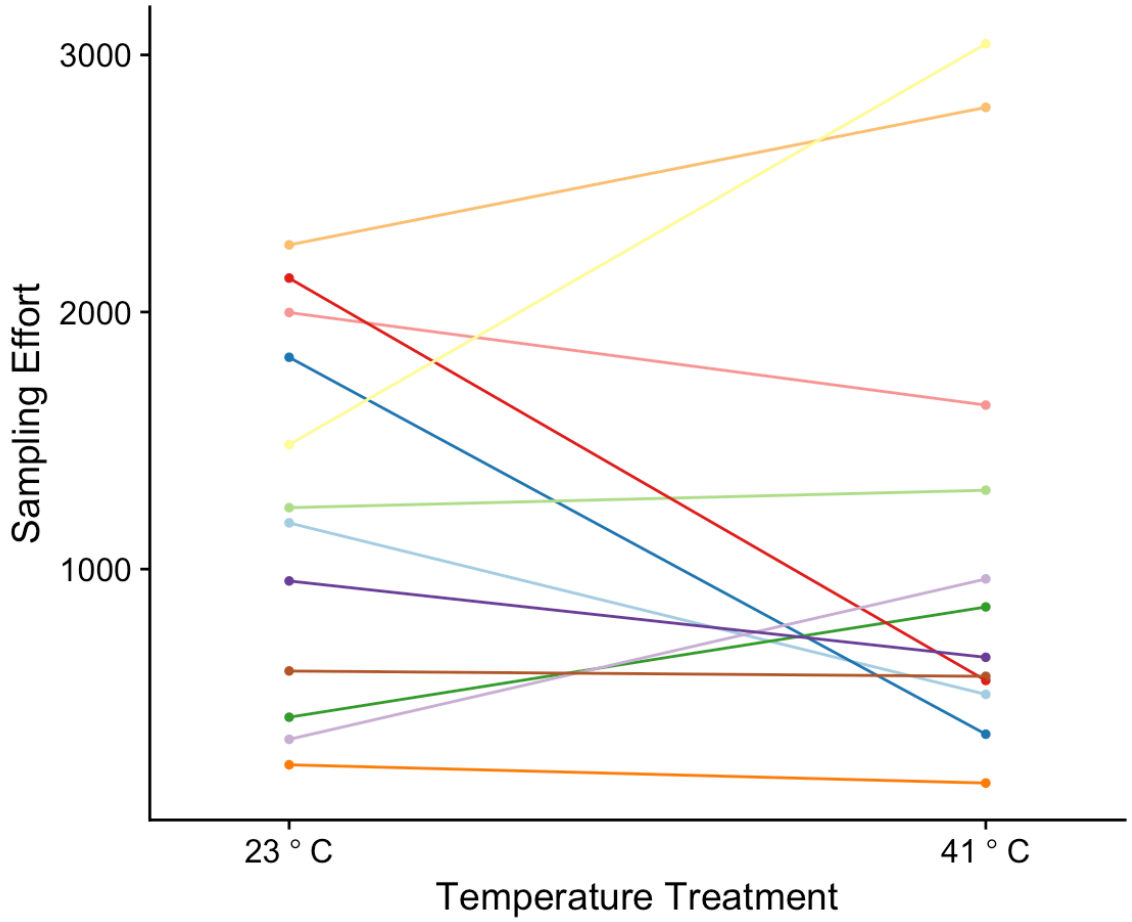

Figure 2-4. Sampling effort between 23°C and 41° trials. Each line represents an individual. Sampling effort was measured by the total number of perch hops in each trial.

DISCUSSION

We found evidence that simulated heat waves influence females' discrimination between male sexual traits. Females ranked conspecific song more highly than heterospecific song while at housing temperature but did not discriminate at temperatures above their upper critical limit. This change in ranking indicates a change in female preference under different temperature treatments. Preference is one factor that influences discrimination; another is sampling effort. Females expended a similar amount of energy sampling songs in both temperature treatments. Taken together, we found a reduction in females' discrimination between male sexual signals at higher temperatures that was explained by a change in preference not by a change in sampling effort.

There are several potential reasons for our pattern of results for how females rank sexual signals. One explanation for our results is that the birds were experiencing stress. Stress can hinder cognition and disrupt brain function (McEwen & Sapolsky, 1995). A study in humans provides some support for this explanation, as subjects made more errors in their jobs at a casting plant while heat stressed (Mazloumi et al., 2014). A recent study found male zebra finches show reduced performance on colour association and cognitive inhibition tasks while heat stressed (Danner, Coomes, & Derryberry, *in prep*). If one considers ranking of sexual signals a cognitive task, then our findings could be explained by reduced cognitive performance under heat stress. This is a plausible explanation because we exposed birds to temperatures above their upper critical limit. Above their upper critical limit, animals experience hyperthermia, or heat stress (Speakman & Król, 2010). Another way in which heat stress could have affected the lack of discrimination between the two stimuli is through loss of motivation. This loss of motivation could be due to suppression of hormone production in the gonads (Rozenboim et al., 2007), or changes in opioid production which has the potential to alter hedonic responses (Pizzagalli, 2015; Riters, 2010). Motivation could also be reduced due to a decrease in social behaviours (Etches et al., 2008). However, if this were the case we would expect to see reduced sampling of songs. Here, it is important to note that the birds had

access to perching on non-experimental perches, yet we still did not observe changes in the sampling of stimuli.

Another possible explanation is that high temperatures distracted females from considering the sexual signals, such that the choice of stimulus became random (Mendl, 1999). Distraction occurs when a challenging stimulus, such as heat, diverts the attention of the animal away from the task (Mendl, 1999). The effect of distraction on mate preference has been illustrated in pipefish (*Syngnathus typhle*) (Berglund, 1993). Typically, male pipefish prefer to mate with smaller females, but choose mates randomly when in the presence of a predator (a stressor and thus considered a distraction) (Mendl, 1999). Work in great tits (*Parus major*) shows that birds also change how they rank signals in the presence of noise, as females respond less to typically preferred lower frequency songs under high noise conditions (Halfwerk et al., 2011). Whether as a result of physiological stress or distraction, we find that females change how they rank male sexual signals when exposed to ambient temperatures above their upper critical limit.

Our second result – sampling effort did not change between the control and hot temperature trials – was unexpected, as we predicted that the birds' activity level would decrease at higher temperatures. Sampling song stimuli requires movement, and movement increases body temperature (Angiletta et al., 2010), which could make it more difficult for animals to thermoregulate. However, one limitation of our study was that thermoregulatory behaviours such as panting and posturing were not measured, meaning that we cannot draw conclusions about how behavioural tradeoffs due to thermoregulation may have affected sampling levels within individuals. Mice under acute heat stress show an increase in activity (e.g. increased escape behaviours) (Harikai, Sugawara, Tomogane, Mizuno, & Tashiro, 2004); however, this explanation is not consistent with our results, as we did not see an increase in activity levels (i.e., the number of perch hops remained the same across control and hot trials). Instead, our results suggest that a reduction in discrimination at elevated temperatures is explained almost entirely by a change in how females ranked songs and not by a change in sampling effort.

Our results suggest that high temperatures in the wild will affect female ranking of male sexual traits. We cannot, however, infer fitness consequences from a reduction in the ability of female zebra finches to discriminate between conspecific songs and the songs of rufous-collared sparrows, because these species do not co-occur. Female finches ranked conspecific song more highly than rufous-collared sparrow songs under housing conditions, indicating that they discriminate between them. Because female zebra finches did not rank such distinct stimuli differently while at temperatures above their upper critical limit, we predict that high temperatures will also affect how females rank the sexual signals of sympatric species and conspecifics in the wild. Reduction in the ability to rank male sexual signals could lead to attempted mating with the wrong species or subspecies (K. S. Pfennig, 1998). Further, effects of heat stress on female preference could also affect an individual's ability to discriminate between conspecifics that differ in quality in a mate choice context. Both contexts could lead to reduced fitness. Additionally, it is possible that male zebra finches decrease song production at high temperatures, as has been shown in other songbirds (Luther & Danner, 2016). However, this is an empirical question. This would compound recently demonstrated fitness consequences of heat stress in this species in which male sperm mobility is reduced at similar high temperatures (Hurley et al., 2018). More experimental studies are therefore needed to test the effects of heat stress on species recognition and mate choice.

Our results also suggest that housing temperatures for zebra finch husbandry are important to consider. A recent review (Beaulieu, 2017) calls into question the temperatures at which zebra finches are typically housed. Environmental variables are often not considered when running experiments with captive zebra finches, for example asking questions about mating behaviour when housing birds at temperatures found in the wild during the non-breeding season (Beaulieu, 2017). Future work should assess how behaviours vary within the thermal neutral zone and contextually relevant temperatures. Here, we demonstrate that temperature does affect mating behaviour, and therefore it is important to house birds at biologically relevant temperatures for typical socio-sexual interactions to occur.

Because thermoregulatory processes are phylogenetically conserved (Angiletta et al., 2010), we also predict similar effects of heat stress on other endotherms. The temperature range for the thermal

neutral zone varies across species, but the order and type of physiological responses to temperatures relative to this thermal neutral zone are similar (reviewed in Huey et al., 2012). For example, heat stress behaviours occur at the upper end of the thermal neutral zone, whether demarcated by 40°C in a desert species such as a zebra finch or by 34°C degrees in a temperate zone breeding species such as white-crowned sparrows (*Zonotrichia leucophrys*) (Wingfield & Farner, 1976). Therefore, our results generate predictions for future studies of heat stress effects on communication behaviours. We predict that a reduction in discriminatory ability among sexual signals will be found at the upper end of the thermal neutral zone for other endotherms. As climate change causes greater temperature increases in temperate zones such as North America, Europe, and Asia than tropical areas (Intergovernmental Panel on Climate Change, 2014), more work on the effect of heat stress on behaviours that affect reproductive success and survival is needed particularly in temperate zone endotherms.

CONCLUSION

Our results indicate that simulated heat waves can reduce the ability of female zebra finches to discriminate between conspecific and heterospecific sexual signals. As temperatures increase worldwide due to climate change, more organisms will experience sub-lethal temperatures outside of their thermal neutral zones. Understanding how organisms behave under these circumstances is key to predicting how species will respond to warming temperatures, particularly in the case of endotherms. Most previous research examining the effects of high temperatures on behaviour has focused on ectotherms (Angiletta et al., 2010; Speakman & Król, 2010). However, recent large-scale mortality events in endotherms (McKechnie & Wolf, 2010; Nilsson et al., 2016) emphasize the importance of modeling how endotherms will respond to climate change (McKechnie et al., 2012). Additionally, behavioural data from ectotherms has been successfully applied to models that map population trajectories in the wake of increasing temperatures (Sinervo et al., 2010). Data from endotherms experiencing sub-lethal effects of high temperatures should be incorporated into models that predict population trajectories and aid in conservation efforts (Boyles et al., 2011; McKechnie et al., 2012).

APPENDIX

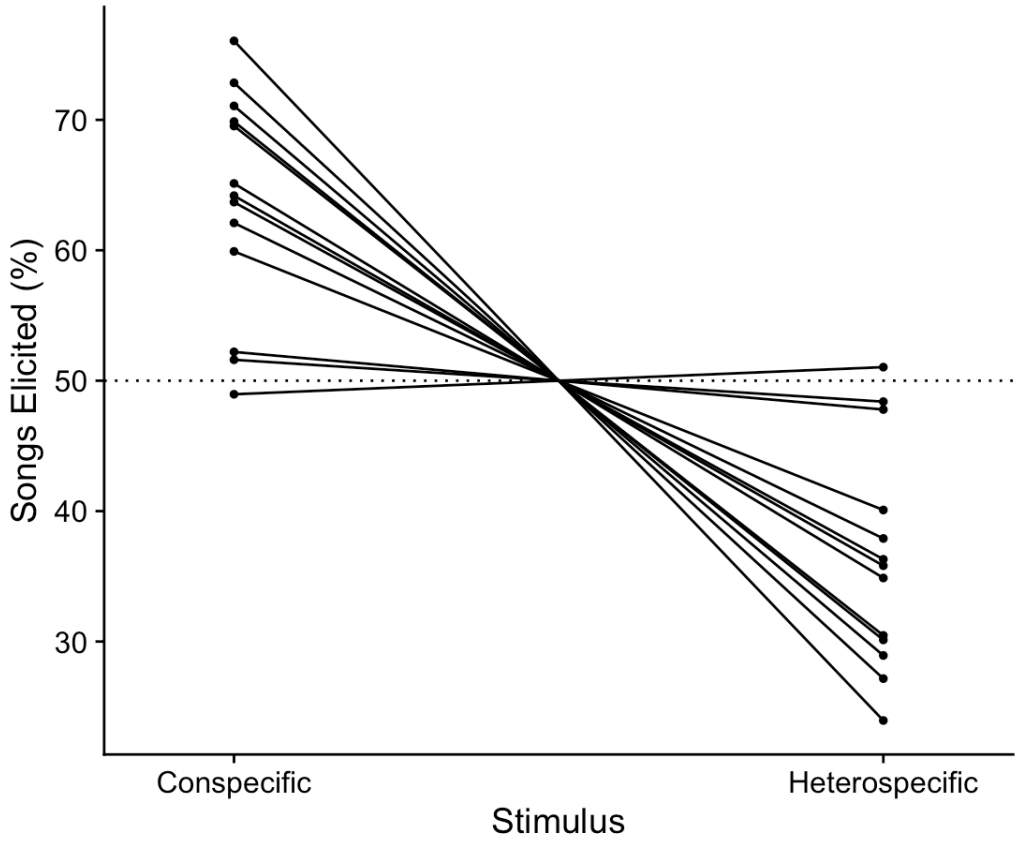

Figure 2-A1: Confirmation of preference for conspecific song. Each line represents an individual bird. The dashed line (50%) represents no preference for either stimulus.

CHAPTER 3

THE EFFECTS OF HIGH TEMPERATURES ON THE SALIENCE OF SEXUAL SIGNALS IN A SONGBIRD

Disclosure Statement: Casey Coomes designed and implemented the experiments contained in this chapter. Casey Coomes is the wrote the manuscript with revisions from supervisor Dr. Elizabeth Derryberry.

ABSTRACT

Animals alter their communication behaviors during thermally challenging events such as heatwaves. Communication behaviors are critical for mate attraction and reproduction in many organisms. Thermal challenges due to high temperatures can affect communication in three fundamental ways: effects on the signaler, effects on the receiver, and effects on the signal itself. Male signalers reduce sexual signal production and female receivers discriminate less against biologically inappropriate sexual signals when exposed to high temperatures. However, we do not know whether thermally challenging temperatures also affect signal *salience*, or the effectiveness of the signal in a functional context. Changes in signal effectiveness can influence how receivers respond to signalers, altering the outcome of mate choice. Here, we test whether high temperatures affect signal salience in a species known to experience declines in communication behaviors under hot conditions: the zebra finch (*Taeniopygia guttata*). We ask whether female zebra finches discriminate between songs produced by the same male at 35°C (within thermoneutrality) and at 43°C (thermally challenging). We find support for our hypothesis that temperature affects signal salience as females discriminate between songs produced at different temperatures. Further, we find that some females prefer 35°C songs while others prefer 43°C songs depending on how males altered their songs in heat. Specifically, males varied in the degree to which they shortened syllable duration under high temperature, with larger males reducing syllable duration more at 43°C. Females discriminated more strongly between songs that changed more under heat. We discuss our results in the context of individual fitness and population persistence as heatwaves become more and more common.

INTRODUCTION

Climate change is causing an ever-increasing number of animals to experience thermal challenges such as heatwaves (Stillman, 2019). In response, animals may change their behavioral strategies, affecting fitness. For example, animals may invest more in behavioral thermoregulation and less in foraging during

thermally challenging hot temperatures. Large mammals such as the *Capra ibex* reduce foraging effort when temperatures are high (Aublet et al., 2009). Southern pied babblers (*Turdoides bicolor*) also reduce their foraging efficiency (du Plessis et al., 2012). High temperatures can also have an effect on mating behaviors. For example, male beetles engage in fewer competitions over females when temperatures are stressful (Berger et al., 2014). Including such behavioral effects of temperature in population trajectory models can help to predict population persistence in the context of climate change (Huey et al., 2012). Reductions in foraging or mating behaviors have helped to predict population declines and extinctions in *Sceloporis* lizards (Sinervo et al., 2010) and in multiple species of desert birds (Conradie et al., 2019). Therefore, collecting such behavioral data is valuable for understanding the impacts climate change will have on animal populations. Here, we test the effects of ecologically relevant high temperatures on communication, which is an essential behavior used to attract and assess mates in many species.

High temperatures experienced during heatwaves can drive changes in multiple aspects of communication. Communication is the transmission of information through the environment from a signaler to a receiver (W. Searcy & Nowicki, 2009). In a mating context, a signaler transmits a signal that advertises attractiveness to a potential mate (the receiver), and the receiver then interprets the information contained in the signal to determine whether or not to mate with that signaler (Andersson, 1994). Higher-than-normal temperatures can influence this process at multiple steps: the signaler, the receiver, and the signal itself (Candolin, 2019). In the case of signalers, thermally challenging high temperatures reduce song rates in song sparrows (*Melospiza melodia*). In contrast, male *Bombina* frogs (Zweifel, 1959), woodhouse toads (Sullivan, 1982), and crickets (Ciceran et al., 1994) increase call rates at high temperature. These opposite responses to heat are likely due to physiological differences between endotherms and ectotherms. Thermally challenging temperatures can also affect receivers. For example, female pied flycatchers, when exposed to a thermal challenge, become less choosy regarding potential mates and are more likely to select a lower quality male (Slagsvold & Dale, 1994). Female pronghorns also exhibit lower sampling effort during heatwaves (Byers, Byers, & Dunn, 2006). In these cases, communication breaks down due to signals and receiver choices shifting in different directions. During a

thermal challenge in ectotherms, communication may be preserved as both the signaler and receiver adjust in parallel with the changing signal, as is the case with treefrogs (Gerhardt, 1978), ultrasonic moths (Greenfield & Medlock, 2007), and treehoppers (Jocson et al., 2019). However, relatively few studies examine all three aspects of communication in endotherms, leaving a critical gap in understanding of the extent to which thermal challenges affect communication behaviors.

Here, we study the effects of thermally challenging temperatures on communication in a well-studied endotherm model, the zebra finch (*Taenoipygia guttata*). Zebra finches are a sentinel species to examine the effects of acute thermal challenges on behavior, as they experience the deleterious effects of heatwaves in the wild (Griffith, Mainwaring, et al., 2016; McKechnie et al., 2012), readily show mating behaviors in captivity (Griffith, Buchanan, & Ed, 2016), and have a well-studied song and mating system (reviewed in Hauber, Campbell, & Woolley, 2010 and Riebel, 2009). Importantly, thermal responses are similar in wild and domestic populations, making it possible to infer the effects of heat on domestic zebra finches to wild populations (Calder, 1964; Marschall & Prinzinger, 1991). Zebra finches have experienced both lethal and sub-lethal effects of high temperatures through mass mortality events (McKechnie & Wolf, 2010) and physiological and behavioral changes, including shifting thermal maxima (C. E. Cooper et al., 2020) and reductions in foraging and social behavior (R. M. Danner et al., 2021; Funghi et al., 2019).

Zebra finch communication, particularly their songs, has been studied extensively. Males sing notes in harmonic stacks called syllables. A combination of several of these syllables is called a motif, and motifs are sung in groups called bouts (Sossinka & Böhner, 1980). Males most often sing two types of song: undirected and directed. Undirected songs are typically sung in social situations, whereas directed songs are most often sung in a mating context (Hauber et al., 2010). Directed songs tend to be longer and higher pitch than undirected songs. Our study focuses on female discrimination among directed songs. There are many studies of female preferences for male song traits (reviewed in Riebel 2009), however there is little consensus about which features of songs females consistently prefer. Female preference in zebra finches is often highly variable due to the fact that females prefer song types that are

familiar (reviewed in Riebel, 2009). However, there is evidence to suggest that females tend to prefer higher song rates, and females who mate with males who have higher song rates produce sexier sons (Houtman, 1992). Additionally, females prefer longer songs (Clayton & Pröve, 1989).

Recent work in zebra finches finds that acute thermal challenges affect male signalers, female receivers, and the signal itself. Male zebra finches sing fewer song bouts when exposed to high temperatures (Coomes and Derryberry, in review). Female zebra finches show less discrimination between conspecific and heterospecific sexual signals (Coomes et al., 2019). Additionally, songs produced under thermally challenging hot temperatures have been shown to have shorter syllable duration (Coomes and Derryberry, in review), which is consistent with work showing that males exhibit shorter syllable and motif duration with increased brain temperature (Aronov & Fee, 2012). Changes in syllable duration may be salient, as female zebra finches tend to prefer songs with longer motifs (Neubauer 1999) and longer note duration leads to longer motifs. However, simply measuring differences in songs produced at different temperatures does not tell us whether the changes in song are biologically relevant. It is necessary to ask the females themselves if these changes in song with temperature are salient.

In this study, we tested the effects of an acute thermal challenge on signal salience in zebra finches. We asked female zebra finches whether they discriminated between songs produced by males at 35°C and at 43°C. Air temperatures of 35°C are within thermoneutrality and air temperatures above 40°C are thermally challenging for zebra finches (Calder, 1964; Christine Elizabeth Cooper et al., 2020). We hypothesized that females would discriminate between songs produced at different temperatures due to changes in signal structure. We did not have an *a priori* prediction regarding which songs females would prefer. If females preferred 35°C songs, then thermal challenges reduce signal salience. If females prefer 43°C songs, then high temperatures may improve signal salience. As individuals vary in thermal tolerance, high temperatures could affect signal salience in some males differently than others. In that case, the differential effects of temperature on signal salience could provide substrate upon which selection could act in wild songbirds.

METHODS

Animal Housing and Care

All females were housed in individual cages (48.26 x 25.4 x 30.48 cm; 19 x 10 x 12 in) in a group housing room in visual and auditory contact. No males were present before or during the duration of this experiment. The temperature in the room ranged from 23°C at night to 27°C during the day. The photoperiod was a 13:11 light dark cycle. All birds had *ad libitum* access to food, water, cuttlebones, and crushed oyster shells. We supplemented diet with fresh vegetables 1-2 times per week. Each cage was lined with newspaper and contained two wooden perches. All housing and experimental protocols were approved by the institutional IACUC (Protocol 2578).

Song recording and stimulus preparation

In order to assay female responses to songs produced at different temperatures, we recorded males producing songs at two different temperatures (see Coomes and Derryberry, In Review). Briefly, we recorded male zebra finches singing inside sound attenuation chambers (IAC mini-acoustical chambers) that had been modified to provide accurate temperature control (±0.3°C) with consistent uniformity (±1°C) across a broad range of temperatures (22-44°C). Directed songs (song types used in mate attraction in zebra finches) were elicited by placing a live female outside of the thermal chamber but in visual contact with the male subject. In a balanced, repeated measures design, we recorded males producing directed songs for 30 minutes at thermoneutrality (35°C) (Calder 1964) and at a physiologically challenging temperature (43°C) (C. E. Cooper et al., 2020; Christine Elizabeth Cooper et al., 2020; Wojciechowski et al., 2020) which induced heat dissipation behaviors in all male subjects (detailed methods see Coomes and Derryberry, in Review).

To select song stimuli, we first reduced the pool of songs by including only those that had introductory notes and at least two motifs. We then selected songs with high signal to noise ratio. From there, we randomly selected songs from each treatment for each male. All songs were then amplitude

normalized using SIGNAL v5 (Beeman, 1999) and volume-adjusted to 65 dB SPL at the chamber center. Stimulus pairs were created using one neutral song and one hot song from the same bird. We created 10 stimulus pairs.

Operant conditioning

We used operant conditioning, an auditory discrimination assay commonly used in zebra finches, to quantify female response to songs. To run the operant conditioning assay, we placed the bird's home cage inside of a sound attenuating thermal chamber and attached two stimulus perches (9 cm long, 6 cm in diameter, made of wood) to the front of each cage, approximately 10 cm above the cage bottom and 25 cm apart. When the bird hopped on the stimulus perch, it triggered a microswitch, which triggered the playback of a song through a speaker. One perch hop triggered a playback of one full song, and if the bird remained on the perch no additional songs were played. We counted perch hops using Sound Analysis Pro (Tchernichovski & Mitra, 2004). In this type of auditory discrimination assay, the song is a rewarding stimulus and females are encouraged to continue hopping on the perches, and the number of times the bird chooses one type of stimulus playback over the other is indicative of the song she prefers (R. C. Anderson, 2009). Operant conditioning results are consistent with other song preference measures in zebra finches such as phonotaxis and copulation solicitation displays (Holveck & Riebel, 2007).

Before beginning trials, we acclimated each bird to the sound attenuation chamber at housing temperature (27°C) for two hours. Then we trained each female on the operant system. In order to train birds, we gave each female a choice between a conspecific and a heterospecific song. In order to pass training, each bird had to hop twice on both perches two days in a row. Females that did not pass training within 4 consecutive days were removed from trials. Seventeen of 20 birds passed the training stage.

For the experimental stage of the trial, each bird was tested using operant conditioning for four consecutive days, alternating morning and afternoon sessions. The morning sessions took place from 930 to 1330, and the afternoon sessions took place from 1400 to 1800. The temperature in the chamber was 35°C. Females heard two different stimulus pairs from two different males, and each heard a different

combination of stimulus pairs to reduce pseudoreplication (Kroodsma, 1990). Each stimulus pair contained songs produced by the same male, one song at 35°C and one at 43°C.

Statistical Analysis

All data were analyzed in R (R Core Team, 2017). In order to determine whether female zebra finches discriminated between songs produced at 35°C and at 43°C, we created generalized linear mixed models with a Poisson distribution (appropriate for count data such as perch hops) using the package lmer (Bates et al., 2015) and model selection using the package AICModAvg (Mazerolle, 2019). Models contained stimulus perch hops as the response variable and temperature treatment, male stimulus, and an interaction between the two as the fixed effects. All models included Female ID as a random effect as females vary in their strength of response to the operant conditioning assay. We compared models using Akaike Information Criterion for small sample sizes (AICc) (Burnham & Anderson, 2004).

From prior work, we knew that males altered syllable duration when exposed to high temperatures (shorter duration at high temps), but that some males were more tolerant of heat than others. Because we randomly selected the song stimuli we presented to females (i.e., not specifically selecting those songs that differed the most under heat), we then quantified the differences in average syllable duration produced by each male at the two different temperatures by subtracting syllable duration at 43°C from that at 35°C. If the resulting syllable duration difference was less than 0 ms, then the bird sang longer syllables in the 43°C treatment relative to the 35°C treatment. If the resulting difference was greater than 0 ms, then the bird sang longer syllables in the 35°C treatment relative to the 43°C treatment. In order to determine whether syllable duration explained variation in female preference strength between treatments and among males, we created a linear model containing a response variable of preference strength and fixed effects of female categorical preference (whether she preferred the 35°C song or the 43°C song), syllable duration difference, and an interaction between the two. To calculate strength of preference, we first calculated preference ratios: the number of perch hops by one female on the 35°C

stimulus divided by the total number of perch hops by that female on both perches. If the preference ratio is greater than 0.5, the female shows a preference for the 35°C stimulus. A preference ratio below 0.5 indicates the female prefers the 43°C stimulus. In either case, a preference ratio that departs from 0.5 shows discrimination between neutral and hot songs. We then subtracted the null preference ratio (0.5) from the demonstrated preference ratio and took the absolute value of this number as the measure of preference strength. We log-transformed strength to normalize the distribution of residuals, required for a linear model.

From prior work, there is some evidence that male mass explains variation in female preference strength (Howell, Anderson, & Derryberry, 2019) and also in behavioral and physiological responses to thermal challenges (Hudson et al., 2013; Kleiber, 1932). For that reason, we then asked if male mass was associated with differences in syllable duration (and possibly therefore preference). To do this, we performed a linear regression on syllable duration difference between temperatures for each male and male mass. For this regression, we calculated the inverse of duration difference in order to normalize the residuals of the model.

RESULTS

Female zebra finches discriminated between songs produced at different temperatures

The model that best explained our pattern of results contained a fixed effect of temperature, stimulus male, and an interaction between the two, including a random effect of female (**Table 3-1**). In other words, some females preferred the songs produced by some males at 43°C, while other females preferred the songs produced by some males at 35°C (**Figure 3-1, Table 3-A1**). Still other females showed a strong preference for songs produced at both temperatures, depending on which male produced the song.

Table 3-1. Model selection for female preference for songs produced at 35°C and 43°C

Model	K	AICc	ΔAICc	Model Likelihood	AICc Weight	Log Likelihood	Cumulative Weight
Temperature*Male	21	1570.39	0	1	1	-754.15	1
Temperature + Male	12	2404.46	834.06	7.68E-182	7.68E-182	-1187.39	1
Male	11	2427.31	856.92	8.36E-187	8.36E-187	-1200.30	1
Temperature	3	2945.96	1375.57	1.99E-299	1.99E-299	-1469.79	1
NULL	2	2969.59	1399.19	1.48E-304	1.48E-304	-1482.70	1

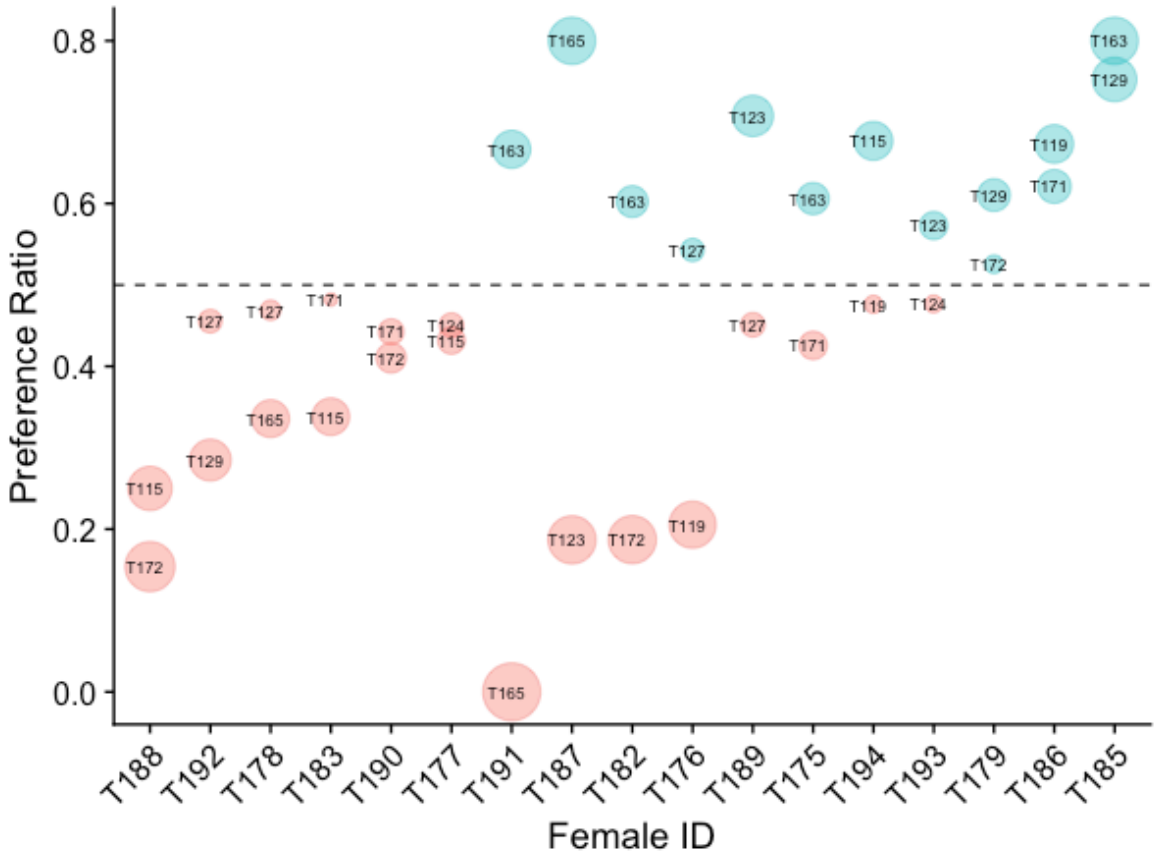

Figure 3-1. Female preference for each stimulus pair. The y axis is the preference ratio for 35°C songs (35°C perch hops/total perch hops). The dashed line at 0.5 represents where the dot would fall if females showed no preference. Blue dots above the dotted line indicate that a female preferred 35°C song for a given stimulus pair. Red dots below the dotted line indicate that a female preferred 43°C songs for a given stimulus pair. Dot size represents the strength of preference for a given stimulus. Dots are labelled with the male ID the stimuli were drawn from.

How males altered their song in heat explained variation in female preference strength

We found a significant effect of the interaction between categorical female preference and syllable duration difference on the strength of female preference (**Figure 3-2, Table 3-2**). Females that preferred songs produced at 35°C more strongly preferred 35°C songs with syllables that were longer than 43°C syllables, and females that preferred songs produced at 43°C more strongly preferred songs that had similar length or longer syllables than 35°C songs. However, the association between strength of preference and change in syllable duration is not as strong for the females that preferred 43°C songs as the it is for the females that preferred 35°C songs. In other words, females preferred the stimulus song with the longer syllable duration within a given pair of song stimuli.

Male size may mediate behavioral responses to heat

We found that difference in syllable duration and male mass are significantly correlated (R^2=0.19, df=32, p=0.005; **Figure 3-3**). Specifically, smaller birds produced songs with shorter syllables at 35°C relative to 43°C, and larger birds produced songs with longer syllables at 35°C relative to 43°C. In other words, larger birds showed greater reduction in syllable duration at 43°C than smaller birds. Only two males produced songs with longer syllables at 43°C, and those males were among the smaller males in terms of mass.

DISCUSSION

We found support for our hypothesis that females discriminate between songs produced at different temperatures. However, some females preferred 35°C songs, while other females preferred 43°C songs. When exploring why females showed differences in their preferences for songs produced at different temperatures, we found that females who preferred 35°C songs showed greater strength of preference for 35°C songs that had longer syllable duration than the 43°C song in that pair. Females that preferred 43°C songs showed greater strength of preference when 43°C songs were longer than, or close to the

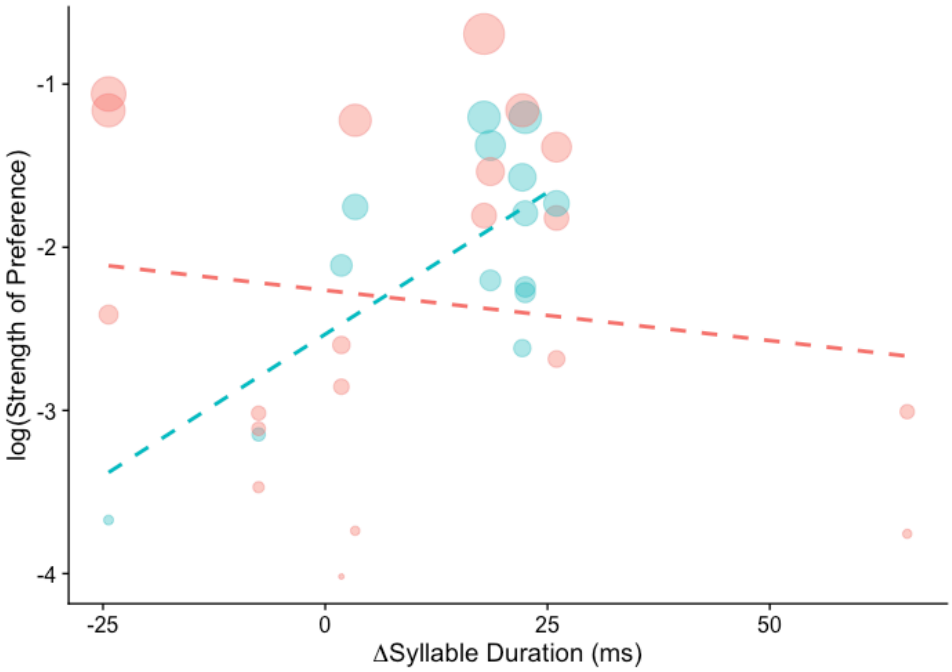

Figure 3-2. Relationship between strength of female preference and change in syllable duration of male song (35°C-43°C). If Δ syllable duration <0, then 43°C songs had longer syllable duration. If Δ syllable duration > 0, then 35° C had longer syllable duration. Blue dots indicate that a female preferred the 35°C songs of a given male. Red dots indicate that a female preferred the 43°C song from a given male. Size of the dot indicates the strength of preference. We observe an interaction between Δ syllable duration and a female's categorical preference. Colored lines represent the line of best fit for each treatment.

Table 3-2. Estimated fixed effects for strength of female preference

Coefficients	Estimate	Standard Error	t value	p value
Intercept	-2.26	0.21	-10.63	p<0.0001
Duration	-0.0061	0.008	-7.67	0.45
35C	-0.27	0.39	-0.69	0.49
Duration*35C	0.041	0.018	2.22	0.034

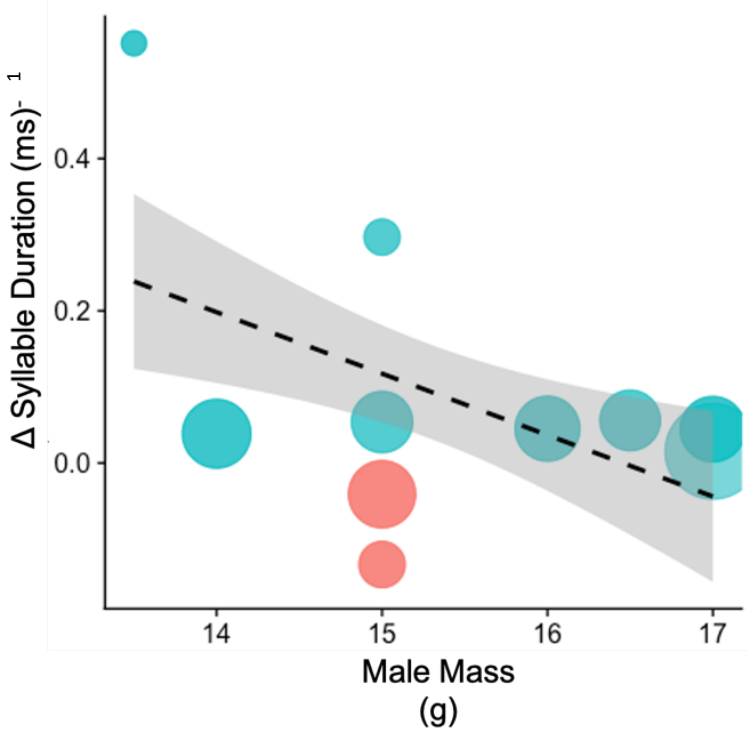

Figure 3-3. Linear regression of the relationship between male mass and Δ syllable duration (35°C-43°C). The color of the bubble represents which song had longer syllable duration. The black dashed line is the line of best fit and the gray area is the standard error. Because the graph shows the inverse of Δ syllable duration, points that are higher along the y axis represent birds whose songs showed less change in syllable duration between the two treatments, and points that are lower along the y axis showed greater change in syllable duration between the two treatments. The size of the bubble represents the magnitude of the difference between the two treatments.

same length as, 35°C songs. We then tested whether change in duration was correlated with features of male morphology that may be important in thermoregulation, such as mass, and found that larger males showed greater reduction in syllable duration between the 35°C and 43°C treatments. This pattern of results suggests that high temperatures affect the salience of male sexual signals. However, the degree and direction of change is dependent on the thermal sensitivity of individual males and individual preferences of females.

Female discrimination between songs produced at different temperatures indicates that thermally challenging temperatures can affect the salience of sexual signals in songbirds. It is well-known that changes in signals due to environmental change affect female response (Endler, 1992). For example, noisy environments can mask the salient features of certain bird songs making females less likely to respond (Francis & Barber, 2013). Visual cues can also become less salient to females when the background does not provide sufficient contrast (J. A. Endler & Thery, 1996). In many circumstances, signalers can show behavioral plasticity and shift the signal that they transmit or switch to a different mode of signaling (audio to visual, olfactory to audio, etc.) (Candolin, 2019). However, animals are less likely to be able to adaptively shift these signals when they encounter a novel environmental change (Candolin, 2019). In this case, the novel environmental change is exposure to thermally challenging temperatures during heat waves.

Changes in signal content as a result of an environmental stressor can have multiple consequences for female receivers. For one, it can render the signal unreliable (Candolin, 2019). A male who sings more or with longer syllables during thermally challenging temperatures, might be more attractive to females. However, it might be more adaptive for the male to redirect energy away from sexual signaling to thermoregulating. If a female mates with this male, she could lower her own fitness by having lower quality offspring. Another potential outcome of changes in signal salience is a disconnect between the direction in female preference and the direction of change of the signal. In other words, signals produced by males could change in ways that make them less attractive to females. In this case, for some

individuals, syllable duration decreases but some females prefer songs with long syllables. This disconnect could result in fewer mating opportunities and reduced fitness for both males and females.

Given that zebra finches show a large amount of variation in thermal responses (Wojciechowski et al., 2020), it is not surprising that we see so much variation among the stimuli produced under heat. In this case, 8 males produced songs with longer syllables at 35°C than at 43°C. Two males sang with longer syllable duration at 43°C than at 35°C. Some males showed a greater decrease in syllable duration from the 35°C to the 43°C treatment than others. This pattern is consistent with prior research testing behavioral responses of zebra finches to thermally challenging temperatures. For example, some male songbirds show greater reduction in motor and cognitive ability and reduction in foraging behaviors than others (R. M. Danner et al., 2021). Some female zebra finches show greater reduction in the ability to discriminate between biologically appropriate sexual signals than others (Coomes et al., 2019). In these studies, a few birds actually showed greater behavioral performance when exposed to high temperatures. For future studies, it will be important to explore the causes behind differences in behavioral thermal response among individuals.

Our results also suggest that body mass may play a role in male behavioral responses to thermal challenges. Larger males showed a greater reduction in syllable duration between 35°C and 43°C. This pattern might be due to larger birds experiencing a greater thermal challenge, because animals with greater body mass have higher basal metabolic rates and therefore have to combat more metabolic heat production (Kleiber, 1932). This pattern has been confirmed in birds, as researchers have shown that birds with greater body mass have higher field metabolic rates (Hudson et al., 2013). Heat retention may have resulted in larger birds experiencing a greater increase in brain temperature, leading to the production of shorter syllables (Aronov & Fee, 2012).

Future studies should examine whether female preference for male zebra finch song is also affected by air temperature. We tested female preference under thermoneutrality (35°C) and this preference could change if females were thermally challenged. Prior work suggests that females zebra

finches may not discriminate between songs under thermally challenging conditions (Coomes et al., 2019). However, work in other taxa suggests that changes in female preference may be coupled with changes in male signals. In other words, females prefer signals produced at cooler temperatures while in cooler environments and prefer signals produced at hot temperatures while in hotter environments (Gerhardt, 1978; Greenfield & Medlock, 2007; Jocson et al., 2019). However, to our knowledge, no studies have addressed this pattern of temperature matching in endotherms. Therefore, how female preference for sexual signals changes under thermally challenging hot temperatures remains a gap in our knowledge of how high temperatures affect communication in songbirds.

Differences in song salience could compound with reductions in behavioral and physiological performance already seen in zebra finches in response to heatwaves. Male zebra finches have been shown to sing less when exposed to high temperatures, which could lead to fewer mating opportunities (Coomes and Derryberry, in review). Male zebra finches also show a reduction in sperm quality when they are exposed to temperatures above 40°C (Hurley et al., 2018). Combined, these effects could lead to reduced reproductive success for males exposed to high temperatures due to fewer mating opportunities and reduced mating success. These behavioral and physiological changes could lead to shifts in the natural and sexual selection act on songbird populations, and potentially lead to population declines.

While zebra finches are a sentinel species for studying the effects of heat, it is pertinent to study the effects of high temperatures in other species, particularly those in temperature regions. There is a large amount of variation in the way that different bird species physiologically respond to high temperatures (Xie, Romero, Htut, & McWhorter, 2017). Zebra finches are well-adapted to high temperatures compared to temperate zone songbirds. For example, the upper critical temperature of the zebra finch is 40°C (Calder, 1964), while the upper critical temperature of the white crowned sparrow (*Zonotrichia leucophrys*) is 34°C (Wingfield & Farner, 1976). Temperate regions particularly in North America, Europe, and Asia are predicted to see some of the largest increases in temperature before the end of the 21st century (Intergovernmental Panel on Climate Change, 2014). Behavioral response to high

temperatures might be more severe in birds that are not well-adapted to high temperatures and are experiencing more severe temperature increases. As climate change continues to cause an increase in the frequency, duration, and intensity of heatwaves (Dosio et al., 2018), understanding how endotherms will behaviorally respond should become a greater priority.

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APPENDIX

Table 3-A1: Estimated Fixed Effects of Female Choice

Fixed Effects	Estimate	Standard Error	z value	p value
Intercept	4.96	0.34	14.35	<0.0001
35 C	-0.18	0.06	-2.76	0.006
T119	-0.23	0.1	-2.28	0.02
T123	-0.83	0.12	-7.04	<0.0001
T124	0.5	0.06	8.31	<0.0001
T127	-0.3	0.1	-3.02	0.002
T129	-0.96	0.1	-9.25	<0.0001
T163	-0.68	0.1	-7.04	<0.0001
T165	-0.31	0.12	-2.6	0.009
T171	0.25	0.09	2.72	0.006
T172	-0.02	0.1	-0.19	0.84
35 C:T119	-0.46	0.11	-3.92	<0.0001
35 C:T123	0.5	0.11	4.4	<0.0001
35 C:T124	-0.01	0.08	-0.11	0.91
35 C:T127	0.07	0.08	0.83	0.41
35 C:T129	0.48	0.09	5.25	<0.0001
35 C:T163	0.78	0.07	10.38	<0.0001
35 C:T165	-0.39	0.11	-3.33	0.0008
35 C:T171	-0.03	0.077	-0.37	0.71
35 C:T172	-0.77	0.08	-9.59	<0.0001

CONCLUSION

My dissertation research demonstrates that high temperatures impact mating communication in an endotherm at multiple steps, meaning that it is crucial to understand how relevant behaviors change as heatwaves become the new normal. I demonstrate that male songbird signalers reduce their production of sexual signals, and these songs can also change structure, specifically syllable duration. Building upon this result, I find that females differentiate between songs produced at different temperatures, meaning that the effects of heat on signal salience are biologically relevant in a mating communication context. However, not all songs males produce demonstrate the same direction of change in syllable duration, which explains variation in female response. This indicates that some birds may produce signals that are more salient under high temperature conditions, which could lead to differential mating success in wild birds. I also find that female songbirds reduce discrimination between biologically appropriate sexual signals when exposed to high temperatures. Reduction in discrimination is explained by a change in ranking of male sexual signals rather than a change in sampling effort. Taken together, these results indicate that heat stress affects songbird signalers, the salience of the signals produced, and discrimination of signals by receivers. The compounding effects of heat at these three stages could have three potential outcomes for songbird populations: evolution through sexual selection, population declines, or even extinctions.

To assess my findings in the broader field of behavioral ecology, I consider what my dissertation research adds to the field as a whole. My dissertation provides the first comprehensive analysis of how temperature affects mate choice from the perspectives of signalers and receivers in an endotherm. The impacts of high temperatures on ectotherm communication and mate choice have been extensively explored over decades. For example, male signaling rates increase during periods of increased temperature in fire-bellied frogs (Zweifel, 1959), woodhouse toads (Sullivan, 1982), and crickets (Ciceran et al., 1994). In many cases, female receivers shift their preferences with temperature in the same direction as males change their signals, as is found in treefrogs (Gerhardt, 1978), ultrasonic moths (Greenfield & Medlock, 2007), and treehoppers (Jocson et al., 2019). The effects of temperature on

endotherm behavior were comparatively ignored because endothermic homeotherms maintain body temperature regardless of environmental temperature conditions. Therefore, the effects of temperature on behavioral performance were considered largely irrelevant. However, with climate change causing an increase in the frequency and intensity of heatwaves, animals experience ecologically unprecedented temperatures outside of the range at which endotherms had adapted to maintain their internal temperatures. My dissertation research contributes to the growing body of evidence that is relevant to study the interaction between temperature and communication and mating behaviors, especially as more animals begin to experience thermally challenging conditions. It is critical to study how temperature affects behavioral performance in different types of endotherms (e.g. mammals and birds), and even variation within the same taxa as many species have evolved different strategies to cope with high temperatures (Fristoe et al., 2015).

To assess my findings in the broader field of communication biology, I consider what my dissertation research adds to how we talk about communication in a changing environment. A growing body of research indicates that climate change (not just temperature) can impact mating behaviors from expression of a trait, transmittance of that trait, and assessment of that trait (reviewed in Candolin, 2019). From the perspective of the signaler – alteration of signals due to temperature can be either adaptive or maladaptive (Candolin, 2019). When male songbirds signal less, then they will likely have fewer mating opportunities as females prefer higher song rates (Houtman, 1992). Males also showed individual variation in song production, with four out of sixteen males increasing song production. However, singing more under thermally stressful conditions isn't necessarily adaptive. Birds could potentially be misappropriating energy toward a costly behavior rather than toward thermoregulatory behaviors. Rather, the adaptive response could be to reduce energy allocated to creating the signal, and redirect it to other behavioral strategies that could be beneficial for survival in hot temperatures (Candolin, 2019). In other words, birds that sing less may be doing more to increase their chances of survival during heatwaves despite the potential cost of reproductive success. However, it is hard to see where a reduction in female discrimination would fit into this framework, as they reduce discrimination but not sampling effort.

Rather, it is possible that in this case females could fall into an evolutionary trap (Robertson, Rehage, & Sih, 2013) by responding to mating signals inappropriately and have reduced mating success despite little change in mating rates.

From a conservation perspective, it is critical to note that changes in mate choice at the individual level could carry over to the population level (Candolin, 2019). In fact, the inclusion of individual mating behaviors in physiology based extinction models improves the accuracy of predictions about population persistence with warming temperatures (Sinervo et al., 2010). In many cases, reductions in mating behaviors lead to population level declines and extinctions, as seen in *Sceloporis* lizards (Sinervo et al., 2010) and in various desert birds (Conradie et al., 2019). However, if there is suitable genetic variation in a population, we may see mating preferences evolve in time with environmental change (D. W. Pfennig et al., 2010). This process also requires physiological and behavioral plasticity. Zebra finches exhibit physiological plasticity when exposed to challenging temperatures (C. E. Cooper et al., 2020; Christine Elizabeth Cooper et al., 2020; Wojciechowski et al., 2020). My dissertation research, along with other behavioral studies, demonstrates that zebra finches exhibit behavioral plasticity in response to rising temperatures (Coomes et al., 2019; R. M. Danner et al., 2021; Funghi et al., 2019; Mariette & Buchanan, 2016; Pessato et al., 2020). However, it is yet to be determined whether the behavioral changes seen in zebra finches are adaptive. The combination of adaptive mate choice decisions, along with other demographic variables such female fecundity and number of breeding individuals could potentially save populations from extinction due to climate change (Candolin, 2019). Notably, therefore, my dissertation research is a critical first step in understanding how temperature affects communication and mate choice so we can make more accurate predictions about how populations will respond to climate change.

When I began my dissertation research in 2015, there were relatively few studies that addressed the impacts of high temperatures on endotherm behavior. Since then, we have developed a much greater understanding of how high temperatures influence songbird behavior, especially in my focal species – the zebra finch. Studies have addressed foraging behavior (R. M. Danner et al., 2021; Funghi et al., 2019), social behavior (Funghi et al., 2019; Pessato et al., 2020), and others address parental behavior (Mariette

& Buchanan, 2016). There remains a gap in understanding of mating behaviors, which my dissertation research addresses (Coomes et al., 2019). There are also a growing number of studies addressing physiological impacts of high temperatures on zebra finches (C. E. Cooper et al., 2020; Christine Elizabeth Cooper et al., 2020; Griffith, Mainwaring, et al., 2016; Hurley et al., 2018; Wojciechowski et al., 2020). In many of these studies, including my own, researchers found individual variation in heat response both physiologically and behaviorally, although this was not measured at multiple temperatures limiting understanding of thermal responses. This highlights an important gap that still exists in our understanding of how songbird populations will respond to climate change.

My dissertation research also highlights a growing need to consider temperature when conducting field and laboratory studies on endotherm behavior. My research demonstrates that temperature does indeed affect songbird communication. My finding that male songbirds reduce song production at high temperatures is consistent with research showing the same patterns in song sparrows (*Melospiza melodia*) (Luther & Danner, 2016). These findings show that it is important to record environmental temperatures when conducting song studies, as temperature could bias results, especially if song recordings are being conducted during a heatwave. Temperature may also be a relevant factor for female or male receivers when conducting playback experiments, as temperature could influence the way receivers interpret signals and thus the manner in which they respond. In addition, it will be important to assess how temperature might bias results of experimental work in the lab. My focal species, the zebra finch, is commonly used for captive studies. However, there is mounting evidence that zebra finches are unintentionally being kept in conditions that don't align with the questions researchers want to ask, and temperature plays a major role in this disconnect (Beaulieu, 2017). Even more notable, these temperature conditions may actually be stressful. For example, birds might have a light-dark cycle indicative of summer, but temperatures associated with winter. On average, most research labs house their finches at 22.8°C, while the lower limit of the zebra finch thermalneutral zone is 27.5°C (Beaulieu, 2017). In the wild, zebra finches most often breed in temperatures above 30°C (Beaulieu, 2017; Griffith, Mainwaring,

et al., 2016; Zann, 1996). Here it is important to note that wild and domestic zebra finches do not differ in their thermal physiology (Calder, 1964; Marschall & Prinzinger, 1991). Because of this, we may see laboratory animals behaving in ways that they would not if they were held in more thermally relevant conditions. It is important for us as researchers to consider the types of questions we ask and whether the conditions in which we house our animals align, especially with captive songbirds such as zebra finches.

FUTURE STEPS

One crucial step in understanding how songbirds will respond to climate change is addressing how high temperatures affect mating communication in wild, temperate species. In North America, almost 30% of birds have disappeared (Rosenberg et al., 2019), and almost two thirds of bird species are at risk of extinction due to rising temperatures (Bateman et al., 2020). Songbirds are particularly vulnerable to high temperatures, and have suffered several mass mortality events as a result (McKechnie & Wolf, 2010). Many avian species experience fitness costs due to high temperatures before their lethal thresholds (Cunningham, Martin, et al., 2013). These fitness costs are often due to physiological and behavioral challenges such as reductions in foraging and changes in nesting behavior (Conradie et al., 2019; Cunningham, Martin, & Hockey, 2015; du Plessis et al., 2012). However, most studies which address these sub-lethal fitness costs do so in desert birds, as these environments are among the first affected by global climate change. Similar behavioral data are severely lacking in temperate zone species. Temperate species have different tolerances and are less well-adapted to high temperatures; compare the upper critical temperature of the zebra finch - 40°C (Calder, 1964) to the upper critical temperature of the white-crowned sparrow - 34°C (Wingfield & Farner, 1976). It is critically important to study how high temperature impacts the behavior of these birds to better predict their persistence in the face of ever-rising temperatures. Our lack of knowledge about how temperate zone species respond to high temperatures is a key gap in our understanding of how songbirds will respond to climate change in the coming decades.

Another crucial step that will further our knowledge of how temperature impacts songbird behavior is to understand the source of the individual variation seen in behavioral responses. Pilot data indicates that zebra finches kept in hot and thermalneutral conditions differ in their regulation of genes related to opioid and dopamine production (Lipshutz et al. in prep). Opioid and dopamine production are related to song production and response to songs (Riters, 2010). It will be important to explore whether variation in the regulation of such gene networks correlate with observed changes in mating behaviors. Additionally, it will be critical to explore whether temperature impacts the regulation of gene networks the same way across species, as birds often have different mechanisms for coping with hot temperatures (Xie et al., 2017).

CAREER TRAJECTORY

My research interest is at the intersection of behavioral ecology and thermal biology. I address questions about how animals respond behaviorally to a warming world, and how these behavioral effects may have consequences for species persistence in the face of climate change. Over the course of my dissertation research, I have become familiar with valuable methodological tools that will help me advance my future research program. The results of my dissertation lay a foundation for exploring further questions about how temperatures influence songbird communication.

As I advance my career, I will ask further questions about how climate change impacts mating and communication behavior in wild, temperate-dwelling species. I will ask questions about how climate change impacts communication at each step (the signaler, the receiver, and the signal), in order to have a more complete understanding of how the entire communication process will change in my focal species. I also plan to expand upon my dissertation research by asking questions about how high temperatures lead to changes in fitness. I will ask whether and to what degree individuals show variation to high temperatures. I will also ask whether the observed individual variation leads to differences in reproductive success. These questions will be critical for understanding how endotherm behavior will facilitate response to as well as change with the ever-increasing pressure of climate change.

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VITA

Originally from Louisville, Kentucky, Casey Coomes discovered her love of animals and exploring the world around her at an early age. After high school, she attended Transylvania University, where she found her passion for scientific research and animal behavior. After graduating in 2015 with a Bachelor of Arts degree in Biology and Spanish Language in Literature, Casey knew that she wanted to continue to study how humans impact the life around them. She then went on to join the Derryberry Lab at Tulane University in New Orleans, Louisiana. Here, she was able to explore questions about how human induced environmental change affects animal behavior. She then transferred with the Derryberry Lab to the University of Tennessee Knoxville, where she completed her dissertation research focused on the effects of high temperatures on sexual communication in songbirds. Casey also participated in collaborative projects with the Danner Lab at the University of North Carolina Wilmington. She will graduate in May 2021 with her Doctor of Philosophy in Ecology and Evolutionary Biology. After graduation, Casey will develop a research program studying the effects of high temperatures on North American songbirds and continue to teach and mentor students both in the classroom and through research.