



High temperatures reduce song production and alter signal salience in songbirds

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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 sexual signalling. For example, songbirds, such as the zebra finch, *Taeniopygia guttata*, use song to attract mates. Here, we test how an acute period of extreme heat affects song production in male zebra finches. We then ask whether female zebra finches discriminate between songs produced at different temperatures. We find that males significantly reduce song output at temperatures that induce heat dissipation behaviours. We also find that males produce song bouts with shorter syllables when thermally challenged. Furthermore, the relative acoustic and structural consistency of songs changes with temperature. Finally, we find that female zebra finches discriminate between songs produced at different temperatures, and the strength of discrimination is associated with changes in syllable duration. Altogether, we demonstrate that heatwave-like conditions can impact communication via alterations in signaller behaviour 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.

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Global climate change is causing an increase in extreme weather events such as heatwaves (IPCC, 2014). Heatwaves become biologically relevant when ambient temperatures exceed thresholds at which species experience fitness costs (Cunningham 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 data related to mating behaviours because they are linked to differential reproductive success, an important driver of evolution (Andersson, 1994). In fact, physiology-based extinction projection models that have incorporated information about how high temperatures affect mating behaviours and reproductive success 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.

Extreme temperatures can affect mating behaviours such as communication, or the transmission of information from a signaller to a receiver (Searcy & Nowicki, 2009). Decades of work on ectotherms (regulation of body temperature depends on external sources) demonstrate that signallers 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, *Bufo woodhousei*, calls increases with temperature (Sullivan, 1982), and male crickets increase chirp rate and reduce interchirp intervals when exposed to high temperatures (Ciceran et al., 1994). In many cases, receivers shift their preferences in tandem with signallers, thus preserving the function of mating behaviours across temperature regimes. For example, this pattern is found in tree hoppers (Jocson et al., 2019), treefrogs (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.

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High temperatures may impede the effectiveness of communication in songbirds as they are particularly vulnerable among endotherms to extreme temperatures (McKechnie & Wolf, 2010). Birdsong 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 signallers and receivers of information contained in song (Riebel et al., 2019). Small songbirds are diurnal, meaning they are active during the hottest parts of the day and more likely to be exposed to thermally challenging temperatures (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 southwestern North America, South Africa and Australia) find that temperatures in these regions are already exceeding thresholds that result in fitness loss and population decline (Conradie et al., 2019; Cunningham et al., 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 temperatures negatively affect either signallers 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 lincolni*, prefer male songs previously heard during cold temperatures over songs they heard under warmer temperature conditions (Beaulieu & Sockman, 2012). Female pied flycatchers, *Ficedula hypoleuca*, spend less time assessing potential male mates in cold temperatures (Slagsvold & Dale, 1994). Female zebra finches, *Taeniopygia guttata*, reduce discrimination between conspecific and heterospecific songs while at hot temperatures (Coomes et al., 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 signallers are less clear.

Experimental and observational work do not always align regarding how temperature affects songbird signallers, although responses to cold temperatures seem to be more consistent. 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, 2008). However, experimental and observational work are not necessarily parallel 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 in zebra finches (Aronov & Fee, 2012), observational work shows that male song sparrows, *Melospiza melodia*, sing less when temperatures are higher (Luther & Danner, 2016). This pattern 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 temperatures. 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 et al., 2016; McKechnie & Wolf, 2010). The number of heatwaves in Australia is increasing (McKechnie et al., 2012), with

heatwaves often lasting several days and average ambient day temperatures above 40 °C (Griffith 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 (~29.5–40 °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 (Cooper et al., 2020), but prior exposure does not change their general physiology (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 behaviours require additional energy expenditure and may interfere with other behaviours (Mitchell et al., 2018) such as song production.

We therefore hypothesized that male singing behaviour varies with air temperature. However, measured differences in songs produced at different temperatures alone does not tell us whether the change in song is biologically relevant. While some work indicates that female zebra finches prefer songs with longer motifs (Neubauer, 1999) (longer note duration leads to longer motifs), it is not known whether the extent of the change in these songs during acute thermal challenge is salient to females. In addition, female songbirds can often detect differences in male songs that researchers cannot (Searcy et al., 1997). While we can make informed predictions about which features of songs are the most salient, females may pick up on a particular aspect of the signal that is not detectable to human observers. Thus, we also measured how female zebra finches responded to songs produced in the 35 °C and 43 °C treatments.

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 (Cooper, Hurley, & Griffith, 2020; Cooper, Hurley, Deviche 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 then asked whether females discriminated between the songs produced in the two treatments using operant conditioning (Holveck & Riebel, 2007). We predicted that males would produce fewer song bouts 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. In addition, we used consistency metrics to compare characteristics of songs such as acoustic accuracy and order of syllables (Tchernichovski et al., 2000) 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. Finally, we predicted that females would discriminate between songs produced in the two treatments. However, because female zebra finches vary widely in

preference among individuals, we had no a priori prediction regarding the direction of discrimination or even whether all females would prefer songs from the same treatment or not. The key question was whether song differences were meaningful to potential receivers. Altogether, we expected to find qualitative and quantitative effects of an acute thermal challenge on singing behaviour 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. Temperatures in the zebra finch range can extend from -5°C to 45°C (Griffith et al., 2016). Zebra finches regularly experience the deleterious effects of heatwaves when breeding in the wild (Griffith 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 et al., 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. There is also 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. Undirected songs are typically sung in social contexts, while directed songs are sung to attract females (reviewed in Hauber et al., 2010). A number of studies demonstrate that female zebra finches prefer males with higher rates of song production (Collins et al., 1994; ten Cate et al., 2006) and songs with longer motifs (Neubauer, 1999) and more consistent frequency modulation (Lauay et al., 2004).

The elements of directed song are extensively characterized (Sossinka & Böhner, 1980). Songs typically comprise 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 we measured the consistency with which males repeated these motifs. There are several ways to measure 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 et al., 2015; Tchernichovski et al., 2000).

Animal Care and Housing

We tested 16 adult male and 17 female zebra finches of unknown age. Birds were obtained from Magnolia Bird Farm in Riverside, California, U.S.A. These are typical sample sizes for a repeated measures experimental design with captive birds (Anderson, 2009; Anderson et al., 2014; Coomes et al., 2019; Gentner & Hulse, 2000). We housed birds in individual cages ($48.26 \times 25.4 \times 30.48$ cm; $19 \times 10 \times 12$ inches) 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 h light:dark cycle, with lights on at 0800 hours and lights off at 2100 hours. Humidity ranged between 45% RH and 65% RH. Males and females associated with each experiment were housed separately in order to ensure that females did not develop a preference for a male's song based on familiarity.

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 $58.4 \times 40.6 \times 35.6$ cm ($23 \times 16 \times 14$ inches) such that a bird's home cage fit within the chamber. 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 ($\pm 0.3^{\circ}\text{C}$) with consistent uniformity ($\pm 1^{\circ}\text{C}$) across a broad range ($22\text{--}44^{\circ}\text{C}$). 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 h at their housing temperature (27°C) to habituate them to light and sound differences. To determine the temperature for the high temperature treatment, we performed a pre-experimental trial to determine temperature at which our population showed heat dissipation behaviours. We placed birds in a thermal chamber at 35°C and then raised the temperature over the course of 1 h until the birds showed signs of behavioural thermoregulation. All birds showed at least one or more heat dissipation behaviours 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 temperature affects song production, we then exposed male zebra finches to 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 et al., 2014; van Dyk et al., 2019). Each bird underwent both temperature treatments, and we alternated the order of treatment to reduce order effects. There were 2 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 min at treatment 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 min 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 (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. To be

counted as a single bout, a vocalization had to have introductory syllables and at least one motif and had to occur at least 3 s 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 of the previously selected song bouts and compared three similarity scores calculated by Sound Analysis Pro: similarity, accuracy and sequential match (Tchernichovski & Mitra, 2004). We did not include songs that contained only 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 versus 35 °C, 43 °C versus 43 °C). This comparison provides insight into the relative consistency with which a bird sings at a given temperature.

Stimulus Preparation for Operant Conditioning

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 v.5 (Beeman, 1999) and volume-adjusted to 65 dB SPL at the chamber centre. Stimulus pairs were created using one 35 °C song and one 43 °C 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 the reward. The number of times the bird chooses one type of stimulus playback over the other is indicative of the song she prefers (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 2 h. Then we trained each female on the operant system. To train the birds, we gave each female a choice between a zebra finch song and a rufous-collared sparrow, *Zonotrichia capensis*, song. To pass training, each bird had to hop twice on both perches 2 days in a row.

Females that did not pass training within four 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 0930 hours to 1330 hours, and the afternoon sessions took place from 1400 hours to 1800 hours. 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.

Ethical Note

All housing protocols and experimental procedures described above were approved by the Institutional Animal Care and Use Committee of the University of Tennessee, Knoxville (IACUC Protocol No. 2578). Birds were acclimated to the facility for 2 weeks before beginning any manipulations. We constantly monitored birds while at the 43 °C temperature using closed-circuit cameras and immediately removed a bird from a 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.

Statistical Analysis

All statistical analyses were performed in R v.4.0.0 (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 et al., 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 for small samples (AICc) using the package AICcmodavg (Mazerolle, 2020). All models contained a random effect of male bird to account for the repeated measures design.

To test whether 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 whether 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 the residuals were normally distributed. For sequential match, we applied generalized linear mixed models with an inverse Gaussian distribution, appropriate for left-skewed data.

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 performed model selection using the package AICcmodAvg (Mazerolle, 2020). 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 identity (ID) as a random effect as females varied in their strength of response to the operant conditioning assays. We compared models using AICc (Burnham & Anderson, 2004).

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. 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 preference strength, 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 35 °C and 43 °C 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.

RESULTS

Song Output Was 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). The next best-fitting model ($\Delta\text{AICc} < 2$) contained a fixed effect of temperature and random effects of treatment order and bird. Birdsong production changed with temperature treatment (generalized linear mixed model, $z = -3.21$, $N = 16$, $P = 0.001$). Consistent with our predictions, we found that male zebra finches produced significantly fewer directed songs at higher temperature ($N = 16$; Fig. 1). During the 35 °C temperature treatment, birds sang an average of seven 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 ($\text{SD}_{\text{bird}} = 0.65$) likely the result of not all birds showing a decrease in song production at high temperature (Fig. 1).

Syllable Duration Was Shorter at High Air Temperature

Syllable duration varied significantly between temperature treatments (linear mixed model, $t = -2.42$, $N = 14$, $P = 0.016$). Consistent with our prediction, syllable duration was shorter in songs produced at higher air temperatures (mean $\pm$ SE: 35 °C: 103.70 ± 60.22 ms; 43 °C: 89.11 ± 61.70 ms; Fig. 2). Here, we also observed a large amount of individual variation ($\text{SD}_{\text{bird}} = 0.62$).

Table 1

Model selection for song count models using Akaike's information criterion for small sample sizes (AICc)

Model effects	K	AICc	ΔAICc	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

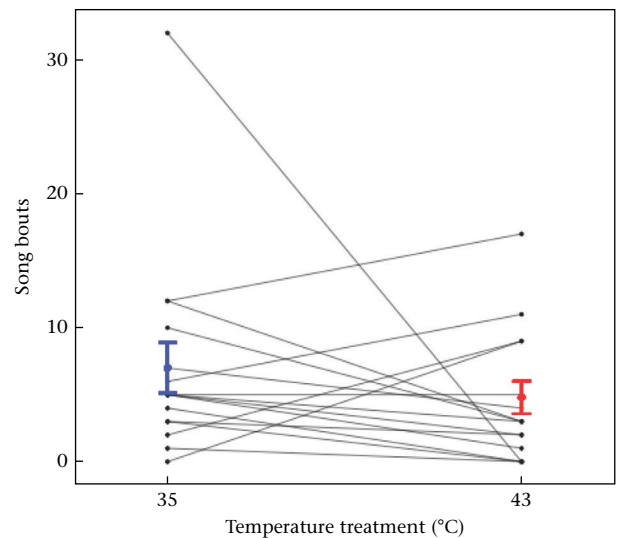


Figure 1. Song bouts produced during 35 °C and 43 °C temperature treatments. Each black line represents an individual bird. The coloured lines represent the population-level mean and standard error within each treatment.

Song Consistency Varied 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 (linear mixed model, $t = -2.59$, $N = 15$, $P = 0.01$). 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 lower air temperatures (35 °C: $89.27 \pm 16.71\%$; 43 °C: $89.84 \pm 14.83\%$). 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: $84.84 \pm 3.87\%$; 43 °C: $85.09 \pm 4.76\%$). Finally, sequential match also varied significantly with temperature treatment ($t = 2.57$, $N = 15$, $P = 0.01$). Consistent with our predictions,

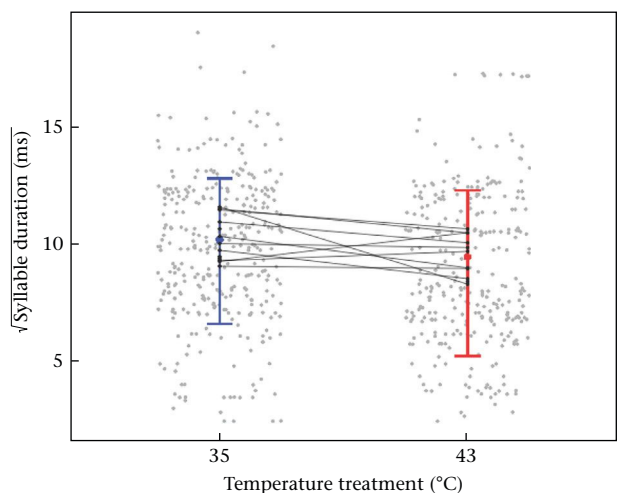


Figure 2. Syllable duration at 35 °C and 43 °C air temperature. Syllable duration has been square-root transformed. Grey dots represent the duration of individual syllables. Black lines represent the change in mean syllable duration for individual birds in each treatment. Coloured lines represent the population-level mean and standard deviation of syllable duration.

Table 2
Model selection for female preference for songs produced at 35 °C and 43 °C

Model	K	AICc	Δ AICc	Model likelihood	AICc weight	Log likelihood	Cumulative weight
Temperature $\times$ 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

we found that songs produced during exposure to higher temperatures had lower rates of sequential match (35 °C: $96.61 \pm 9.83\%$; 43 °C: $93.81 \pm 8.98\%$). Overall, songs produced at higher temperatures were more similar and more accurate but less consistent in sequential match than songs produced at lower temperatures.

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 2). 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 (Fig. 3, Appendix, Table A1). Still other females showed a strong preference for songs produced at both temperatures, depending on which male produced the song.

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 (Fig. 4, Table 3). 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 longer syllables relative to 35 °C syllables. However, strength of preference for 43 °C was not as closely associated with change in syllable duration as the strength of

preference for 35 °C songs. In other words, females tended to prefer the stimulus song with the longer syllable duration within a given pair of song stimuli.

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, and past experimental studies, syllable duration was shorter at hotter temperatures. This variation in syllable duration was biologically meaningful to females. Females discriminated between songs produced at different temperatures, and the strength of this discrimination was explained by variation in syllable duration. Altogether, our results provide strong experimental evidence that short-term exposure to extreme but ecologically relevant high temperatures affects song production and signal salience 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 domestic chickens, *Gallus domesticus*, heat stress has been shown to influence the production of gonadal hormones (Rozenboim et al., 2007). While a direct relationship between heat and androgen levels has not been found in male zebra finches, there is some evidence that an acute heat challenge does affect testes, where androgens are produced, as sperm quality decreases when temperatures reach above 40 °C (Hurley et al., 2018). A second potential mechanism for this change is a

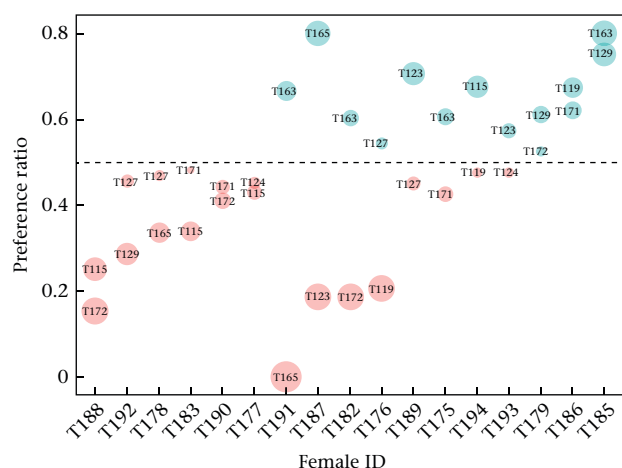


Figure 3. Female preference for each stimulus pair. The Y axis is the preference ratio for 35 °C songs (35 °C perch hops/total perch hops). The dashed line at 0.5 represents where the dot would fall if females showed no preference. Blue dots above the dotted line indicate that a female preferred 35 °C 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.

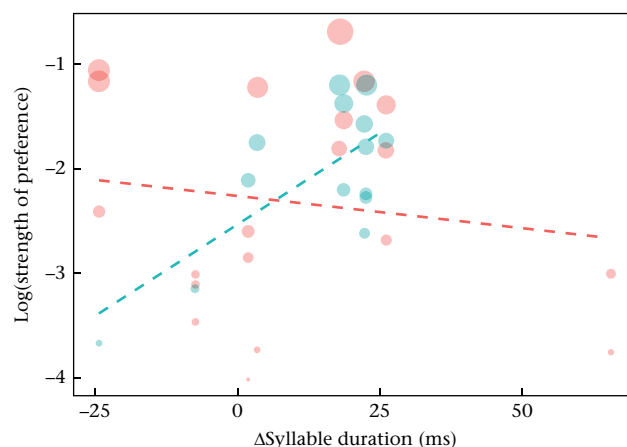


Figure 4. Relationship between strength of female preference and change in syllable duration of male song (35–43 °C). If Δ syllable duration < 0 , then 43 °C songs had longer syllable duration. If Δ syllable duration > 0 , then 35 °C had longer syllable duration. Blue dots indicate that a female preferred 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. Coloured lines represent the line of best fit for each treatment.

Table 3
Estimated fixed effects for strength of female preference

Coefficients	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	−2.26	0.21	−10.63	<0.0001
Duration	−0.0061	0.008	−7.67	0.45
35 °C	−0.27	0.39	−0.69	0.49
Duration × 35 °C	0.041	0.018	2.22	0.034

reduction in dopamine or opioid production. Dopamine production and opioid release are heavily involved in the motivation to sing and make singing rewarding (Riters, 2010). However, whether high temperatures affect the production of androgens, dopamine or opioids is a separate empirical question.

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. Domestic chickens have been shown to decrease social interactions at high temperatures (Etches et al., 2008). Another potential explanation is that males experience trade-offs in terms of time or energy budgets between song production and heat dissipation behaviours. Birds have already been shown to undergo behavioural trade-offs between thermoregulation and foraging behaviour (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 expend more energy thermoregulating and performing heat dissipation behaviours such as panting than they do when 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 et al., 2012), and females who choose males with higher song rates have higher-quality offspring and sexier sons (Houtman, 1992). Furthermore, 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 heatwaves.

In addition to reduced song production, we also found that certain song features changed under high temperatures. These changes in song features may explain why females discriminated between the two different song types. Males altered syllable duration when exposed 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 become shorter as brain temperature rises from 38 °C to 43 °C (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 behavioural output of the birds, previous work on zebra finches did find that male zebra finches at 44 °C experience an increase in body (i.e. cloacal) temperature by ~3.5 °C (Calder, 1964). No matter the mechanisms underlying the decrease in syllable duration, production of shorter syllables may have consequences for mate choice. Female zebra finches tended to prefer 35 °C songs from males that had longer average syllable duration during the 35 °C treatment than during the 43 °C treatment.

This pattern is consistent with what is known about female preference in zebra finches, as 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. Syllable duration can also be indicative of age, and while females tend to prefer more mature males, it is unknown whether females utilize this information when choosing a mate (reviewed in Riebel, 2009). Because female zebra finches discriminated between songs produced at different temperatures, males who undergo changes in song structure due to heat stress may be less capable of attracting potential mates. However, the degree and direction of change depend on the thermal sensitivity of individual males and individual preferences of females.

The differences in the consistency with which zebra finches produced songs in the different temperature treatments could be explained by temperature's effect on the brain or on motor coordination. Birds varied in their overall song consistency 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 neurological or anatomical constraints on song production) when singing at 35 °C. These demonstrated changes in song accuracy are consistent with previous studies showing that brain temperature is associated with changes in 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 (Danner et al., 2021).

We also observed individual variation in behavioural 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 behavioural performance in heat has also been noted in other studies of the effects of heat on behaviour, including female mate choice (Coomes et al., 2019) and foraging behaviour (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 have larger thermal windows (increasing their ability to dissipate heat) (Tattersall et al., 2009), larger mass (produce more metabolic heat) (Speakman & Król, 2010) or differential expression of gene networks related to metabolic rate (Stager et al., 2015), water balance (McCormick & Bradshaw, 2006) or singing behaviour (Maney et al., 2005). The question of why individuals varied in their behavioural 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 signal salience 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 behaviours (Griffith 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 also forage less often during periods of high temperature, reducing overall food intake and social interaction (Funghi et al., 2019). In

addition, zebra finches produce a unique call that aids thermoregulation when exposed to high temperatures, known as 'vocal panting' (Pessato et al., 2020). Temperature also affects 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 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. 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 temperate regions. There is large variation in the way that different bird species physiologically respond to high temperatures (Xie et al., 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 (IPCC, 2014). Behavioural 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 behaviourally respond should become a greater priority.

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Appendix

Table A1

Estimated fixed effects of female choice

Fixed effects	Estimate	SE	z	P
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

T119–T172 denote bird IDs.