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Thermoregulatory Behavior in Zebra Finches (Taeniopygia guttata) in a Lab-Controlled Setting

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To the Graduate Council:

I am submitting herewith a thesis written by Clara Howell entitled "Thermoregulatory Behavior in Zebra Finches (Taeniopygia guttata) in a Lab-Controlled Setting." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

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Thermoregulatory Behavior in Zebra Finches (*Taeniopygia guttata*) in a Lab-Controlled Setting

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ABSTRACT

As heat waves increase in length, duration, and intensity and average temperatures continue to rise globally, hot temperatures are predicted to have increasingly negative effects on animal populations. The ecology and physiology of songbirds make them particularly susceptible to these rising temperatures, as can be seen in mass-mortality events of desert birds during heat waves. While it is increasingly important to understand how heat affects animal populations, many aspects of the heat response are underdescribed, and it is unknown how various levels of response, such as behavioral and cellular, are integrated. In this study, we characterized the behavior of a model songbird, the zebra finch (*Taeniopygia guttata*), at temperatures below (27°C), within (35°C), and above (43°C) its thermoneutral zone of 29.5 – 40°C. We characterized thermoregulatory (panting and piloerection) and self-maintenance (eating, drinking, grooming, fluffing, and moving) behaviors during a fifteen-minute period after 2 hours of temperature exposure. Even with a relatively small sample size (6 individuals per treatment group), we found significant increases in panting behavior in the hot treatment group and significant increases in piloerection behavior in the cold treatment group. Further research is required to determine whether temperature affects non-thermoregulatory behaviors. Our results provide a starting point for investigating how lab and wild observations of thermoregulatory behavior differ, and for comparing heat responses across systemic levels.

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PART 1: INTRODUCTION

As heat waves globally become longer, more intense, and more frequent (Gerald and Tebaldi 2004; Perkins et al. 2012; Mann et al. 2017), heat is projected to have an increasingly negative impact on animal populations (Angilletta 2009; Mckechnie and Wolf 2010; Hoffmann and Sgró 2011). These negative impacts include highly visible mass mortality events, such as the death of thousands of budgerigars (*Melopsittacus undulatus*) and zebra finches (*Taeniopygia guttata*) during the 1932 and 2009 heat waves in Australia (Mckechnie and Wolf 2010; Sherwood and Huber 2010). In addition to these lethal effects are less visible cognitive and behavioral effects (du Plessis et al. 2012; Lee et al. 2015; Coomes et al. 2019) that may have long-lasting impacts on animal condition (Gardner et al. 2016), fertility (Walsh et al. 2019) and, by extension, population persistence (Sinervo et al. 2010). While knowledge of lethal heat limits allows for some predictions of population movement and decline in response to changing heat patterns (Mckechnie and Wolf 2010; Sinervo et al. 2010; Levesque et al. 2016; Albright et al. 2017), much less is known about cognitive and behavioral effects of heat and how they may contribute to these processes, particularly in endotherms (Khaliq et al. 2014).

One important aspect of studying the sub-lethal effects of heat stress is characterizing the thermoregulatory behavioral response of animals at different temperatures (Wingfield et al. 2017). Thremoregulatory behaviors are both an animal's first defense against heat and, if they interfere with self-maintenance or reproductive behaviors, one of the first fitness consequences of raised temperatures. For instance, when temperatures rise, animals initially move to cooler microclimates (e.g. shade) to lower their immediate ambient temperature (Harikai et al. 2004; Funghi et al. 2019). However, moving to microclimates or staying close to a water source can limit foraging opportunities (Funghi et al. 2019). Animals also use postural changes to increase heat loss through convection (Angilletta 2009), but such thermoregulatory behavior can interfere with the efficiency of foraging (du Plessis et al. 2012). While some animals may be able to cope with a temporary deficit of calories, this is more difficult in small animals with high metabolisms, like songbirds (Mckechnie and Wolf 2010), and may explain some of the lasting negative impacts of heat waves on bird populations (Gardner et al. 2016). Establishing an animal's baseline of thermoregulatory behaviors in a lab-controlled setting is important as it allows for comparison with wild behavioral observations at the same temperature, to determine whether wild animals under energy constraints are budgeting the expected amount of time on thermoregulation and whether the types of calorie intake reductions seen in the wild (du Plessis et al. 2012) are also observed when foraging is not energetically costly.

The effectiveness of thermoregulatory behaviors may depend on an animal's morphology and cellular response, though the degree to which these three factors (behavioral response to heat, cellular response to heat, and morphology) interact is largely unknown. Morphological traits, such as bills in birds, can facilitate heat loss through convection (Angilletta 2009; Tattersall et al. 2018), and may result in fewer behavioral trade-offs in heat. For instance, one study that found larger-billed birds were able to sing more at high temperatures (Luther and Danner 2016). The effectiveness of the cellular response to heat stress, which includes heat-shock proteins, protein manufacturing proteins, and other cell-repair mechanisms (Evgen'ev et al. 2014) may also limit

the degree to which an animal needs to behaviorally thermoregulate by raising the temperature at which cellular damage occurs. Establishing an animal's baseline of thermoregulatory behaviors in a controlled lab setting is also important as it allows for the integration of behavioral information with morphological and cellular response (for instance, transcriptomic) information.

In this study, we sought to establish a thermoregulatory behavioral baseline in the zebra finch, a model organism of cognition (Healy et al. 2010) and, increasingly, thermoregulation (Beaulieu 2016; Coomes et al. 2019; Funghi et al. 2019). The zebra finch is an ideal model organism for thermoregulation in heat because it is endemic to hot, arid regions in central Australia (Zann 1996) and as such provides a conservative estimate of the effects of increasing ambient temperatures on avian populations. Studying the effects of heat on passerine species is also important and timely because certain aspects of their physiology and ecology make them particularly succeptible to heat waves (Mckechnie and Wolf 2010; Khaliq et al. 2014). For instance, as mentioned earlier, songbirds tend to have high energetic needs in proportion to body mass, tightening energetic constraints (Mckechnie and Wolf 2010). They are also particularly susceptible to dehydration, as evaporative water loss can exceed 5% of their body weight per hour (Wolf and Walsberg 1996). Ecologically, many songbird species (at least 15%; Khaliq et al. 2014) are already endemic to regions that are frequently hotter than the upper limits of the thermoneutral zone (the range of temperatures at which an animal spends the least energy thermoregulating; Angilletta 2009). In addition, because songbirds are diurnal, they are most active during the times of day when temperatures are highest, and are able to make limited use of microclimates for cooling (Mckechnie and Wolf 2010; Funghi et al. 2019).

The zebra finch's thermoneutral zone (TNZ) is 29.5 - 40°C (Calder 1964). We measured thermoregulatory behavior below (27°C), within (35°C), and above (43°C) the thermoneutral zone in order to establish a lab-controlled thermoregulatory baseline for this species. Although our initial research questions concerned the response of the zebra finch to heat, studying behaviors below the zebra finch thermoneutral zone is important because it allows comparison of behavior at colder and hotter temperatures, and because, in contrast to their wild counterparts, domestic zebra finches are frequently housed at temperatures that require them to increase their metabolism to raise body temperature (Beaulieu 2016).

Overall, we hypothesized that birds would invest more time in thermoregulatory behaviors at temperatures outside of their thermoneutral zone, such as panting to keep cool and piloerection to keep warm. We further hypothesized that an increased investment in thermoregulatory behaviors would cause changes in self-maintenance and fitness-related behaviors, such as eating, drinking, and grooming. We collected data on a number of behavioral states (panting, piloerection, and neutral posture), animal position within the cage (recorded as "time spent aloft", or time spent either on perches or cage walls), and a list of additional behavioral events (eating, drinking, grooming, fluffing feathers, and moving, which was defined as any time both feet left the ground).

We predicted that at 27°C, which is below the zebra finch thermoneutral zone, we would see an increase in warming thermoregulatory behaviors. In birds, this includes increasing metabolism and activity (Marsh and Dawson 1989), erecting feathers to increase plumage insulation (Stettenheim 1972; Marsh and Dawson 1989), and covering sites of heat loss (e.g. legs, beak, and eyes; Midtgard 1984; Marsh and Dawson 1989).We thus predicted that we would see increased piloerection ("puffing" feathers), activity, and eating in birds in the cold treatment. We

predicted that at 35°C, within the thermoneutral zone, zebra finches would show the fewest thermoregulatory behaviors (i.e. spend the least amount of time thermoregulating). Because they would spend less time thermoregulating, we predicted that birds in this treatment would show more activity, particularly in relation to the hot group. We further predicted that at 43°C, which is above the zebra finch thermoneutral zone, we would see an increase in cooling behaviors. Avian species tend to shed heat through evaporative cooling (i.e. panting; Richards 1970) and by increasing blood flow to sites of heat exchange (Daghir 2008; Josipovic and Iudwig 2012) such as the legs and beak (Tattersall et al. 2018). This often entails wingspreading and standing tall postural changes (Zann 1996; Funghi et al. 2019), but the position of our cameras did not allow reliable collection of those data. Other avian species have been known to reduce activity level in heat to avoid unnecessary thermogenesis (Daghir 2008), though the extent to which this is true for zebra finches is unknown. We therefore predicted that in zebra finches we would see an increase in panting and drinking water, and a reduction in overall activity level. Because morphology has been linked to thermoregulation in other species of birds (*Geospiza spp.*, Greenberg et al. 2012; *Melospiza melodia*, Luther and Danner 2016; Tattersall et al. 2018), we predicted that the size of thermoregulatory windows (beak surface area and tarsus length) would be inversely correlated with cooling behaviors. We further predicted that heavier birds would spend more time on cooling behaviors in the Hot treatment, and less time on warming thermoregulatory behaviors in the Cold treatment, as fat is thermally insulating (Angilletta 2009). We collected data on these behaviors at two different time points: two hours after reaching maximum temperature, and immediately before cessation of the temperature manipulation, approximately five hours after reaching maximum temperature. The presence and extent of thermoregulatory behaviors were then compared between temperature groups and across time points, in order to ask how zebra finch behavior changes depending on temperature in relation to the thermoneutral zone.

PART 2: METHODS

Subjects and housing conditions:

We obtained 24 adult male zebra finches from Magnolia Farms Avian Breeder (Anaheim, CA). Prior to purchase, birds were housed in heated outdoor aviaries that experience typical maximum temperatures of 27.7°C in the summer and typical minimum temperatures of 8.3°C (mitigated by heat lamps) in the winter (based on Orange County, CA climatological data; NOAA). After purchase, birds were housed in a room with an average daily temperature of 24 – 25°C, with a high of 26°C and low of 23°C.

Birds were housed in a group cage for four days after arrival, after which they were weighed using a Pesola scale, nail-trimmed, and re-housed in individual cages. Individual cages were made of wire and measured 48cm x 25cm x 30cm. Each cage had two perches, one cuttlebone, one food dish, and one water dispenser. Food and water provided *ad libitum*. Enrichment was provided weekly in the form of a water bath, shredded paper, and millet. Room was illuminated on a 13:11 light-dark cycle. Housing conditions were approved under University of Tennessee Knoxville IACUC Protocol 2578.

Temperature manipulations:

Temperature manipulations occurred in 6 sound attenuation chambers (Industrial Acoustic; MAC 1) that were modified to allow for temperature control by UT engineers. Standard environmental chambers are too loud to study avian behavior, and so engineers at UT modified the sound attenuation chambers to provide accurate temperature control (±0.3°C) with consistent uniformity (±1°C) across a broad range of temperatures (22—44°C). All chambers had vents and fans that allowed for continual circulation of air, temperature probes to monitor chamber temperature, and two cameras placed in the upper rear corners of the chamber to record behavior. Chambers fit one individual cage with enough room on the sides for two cameras and enough room above for the placement of a temperature probe. To reduce chamber effects, chambers were assigned to each treatment group on a rotating block schedule such that each chamber was set to each temperature treatment at least once.

Temperature manipulations consisted of 36 hours of acclimation followed by exposure to one of three temperatures: 27°C (3 degrees below the zebra finch thermoneutral zone; Calder 1964), 35°C (in the middle of the zebra finch thermoneutral zone; Calder 1964), or 43°C (3 degrees above the zebra finch thermoneutral zone; Calder 1964).

The purpose of the acclimation period was to allow birds to adjust to the thermal chambers, in which birds are visually and acoustically isolated from neighbors. In this way we attempted to isolate the observed behavioral effects to those caused by temperature changes, and not by stress in reaction to a novel setting. On day 1 of acclimation, individual cages were moved into the thermal chambers at 12pm. Thermal chamber doors were closed initially but reopened from 2pm – 4pm to allow birds to hear one another. On day 1, the internal temperature of the chamber was

consistent with room temperature outside, 22°C. On day 2 of acclimation, temperatures remained at room temperature until 11am, at which point it was raised to 27°C. Temperatures stayed at 27°C until 3pm, at which point chambers were turned off and temperatures dropped to 22°C again. Day 3 consisted of experimental temperature manipulations. Because behavioral observations were paired with tissue analysis that required time for collection, manipulations were staggered at 15 minutes apart throughout the day, typically beginning at 9am and ending at 4pm. The length of manipulation depended on the temperature, as chambers took longer to reach higher temperatures. For the Hot (43°C) group, manipulations lasted 6 hours, including the time required to reach 43°C. For the TNZ (35°C) group, manipulations lasted 5.5 hours, including the time required to reach 35°C. For the Cold (27°C) group, manipulations lasted 5 hours, including the time required to reach 27°C.

Video recording:
Two cameras (Logitech webcams) were placed in the upper-right and upper-left corners of each thermal chamber, allowing for almost complete coverage of the chamber. Recording occurred during the full extent of the temperature manipulation. Videos were cut and different views synced together using iMovie.

Behavior analysis:
Behavior was sampled at two 15-minute increments: (1) two hours after the chamber reached the set point (27°C, 35°C, or 43°C), and (2) during the final 15 minutes before tissue collection. Because chambers varied slightly in the time they took to reach the set point, there is slight individual variation in times between sample points 1 and 2. On average, however, sample 2 occurred 2 hours and 57 minutes after the start of sample 1 (standard deviation = 17 minutes).

After videos were cut into the required 15-minute intervals, they were randomized in order and renamed by an independent observer so that scoring would be done blindly. All videos were scored by the same individual to maintain consistency in scoring. Videos were scored using the Animal Behaviour Pro app (Nicholas Newton-Fisher) using an ethogram for normal and thermoregulatory behaviors (see Appendix A).

Morphological measurements:
Morphological measurements were taken post-mortem immediately after the temperature treatments ended. Measurements taken were mass, wing length, tarsus length, bill length, bill depth, and bill width. Bill surface area was calculated by estimating the bill as a cone (Luther and Danner 2016): bill length x π x (bill width + bill depth) / 4.

Statistical analysis:
Behaviors were categorized as states or events. Both types of behavior were compared across treatments using one-way ANOVAs, followed by a post-hoc Tukey's honest significant difference test for multiple comparisons. Comparisons between the first time point (2 hours) and the second time point (3.75 hours) were done using paired t-tests. Correlations within treatments between behaviors and morphological measurements were tested using linear models. Although initially

treatment groups had 8 individuals, camera malfunctions made only 6 in each group available for analysis. Thus analysis was performed on 6 birds in each treatment group. All stats were performed in R (R Development Core Team 2016).

PART 3: RESULTS

Behaviors did not differ with exposure time to heat

We did not find any difference in behaviors (states or events) with exposure time to heat. In other words, the presence and expression of behaviors after two hours of heat was similar to that expressed after four hours of heat. The first time point did not differ significantly from the second time point in any states measured: piloerection (t=-0.10; df=17; p-value=0.919), neutral posture (t=0.62; df=17; p-value=0.529), panting (t= -0.61; df=17; p-value=0.551), or time aloft (t= -0.37, df=17, p-value=0.712). The first time point also did not differ significantly from the second time point in any events measured: eating (t= -0.81, df=17, p-value=0.428), drinking (0.72, df=17, p-value=0.480), fluffing feathers (t= -.84, df=17, p-value=0.412), grooming (t= -0.250, df=17, p-value=0.807), or moving (0.68, df=17, p-value=0.507). The effect size at which we can reject the null hypothesis with 0.95 confidence is 0.902, so we can be reasonably confident that there is no difference in behaviors between the groups or that the effect size (difference of the means divided by the pooled standard deviation) is less than 0.902. As there was no difference between these two time points, we report analysis of the first sample, as it was determined by time after temperature conditions were reached instead of time before tissue collection. Analyses of the second sample and pooled samples can be found in Appendix C.

Birds exhibited significant differences in thermoregulatory behaviors across treatments

The three groups differed significantly in time spent in each of the three behavioral states: panting, neutral posture, and piloerection (feathers erect) (See Figures B.1, B.2, and B.3). Consistent with predictions, birds in the Cold (27°C) group spent, on average, 0% of their time panting (Fig. B.1), 46.7% of their time in a neutral posture (Fig. B.2), and 53.3% of their time piloerect (Fig. B.3). Birds in the TNZ (35°C) group spent an average of 6% of their time panting (Fig. B.1), 88.7% of their time in a neutral posture (Fig. B.2), and 5.2% of their time piloerect (Fig. B.3). Also consistent with predictions, birds in the Hot (43°C) group spent an average of 75.2% of their time panting (Fig. B.1), 24.8% of their time in a neutral posture (Fig. B.2), and 0% of their time piloerect (Fig. B.3). See Table A.1 for details. We did not find a significant difference among temperature treatment groups in time spent aloft in the cage, although it was close to significant (F-value=3.34, p-value=0.063) with birds in the Hot group tending to spend more time on the ground than birds in the Cold group (difference= 409.07 seconds, Tukey post-hoc: p-value=0.06). Because multiple behavioral state comparisons were made on the same data set, the alpha on each ANOVA was corrected with a sequential Bonferroni, to 0.0125 initially. The results on panting, neutral posture, and piloerection stayed significant.

Birds did not change in other behaviors, such as eating, between temperature treatments

Of the behavioral events surveyed, only one, “fluffing”, was significantly different between treatments (F-value=4.36, p-value=0.0322), and this result was not significant with the adjusted alpha of 0.01. We did not find significant differences in the number of times that birds drank water, ate seeds, groomed or moved between temperature treatment groups (Table A.1). However, power to detect even a relatively large effect size (0.4) with our given sample sizes is only 0.26, so we

cannot exclude the possibility that temperature affected these behavioral events. In particular, we found that birds tended to eat more at colder temperatures (mean=14.0 instances in the sampling period) and less at warmer temperatures (mean=10.17 at 35°C, 5.0 at 43°C), groomed more outside of their thermoneutral zone (mean=10.83 at 27°C and 13.17 at 43°C, compared to 3.67 at 35°C), and moved more in their thermoneutral zone (mean=195.2 at 35°C, compared to 162.5 at 25°C and 82.3 at 43°C), all of which are behaviors consistent with our predictions. See Table A.1 for behavioral events.

Morphology did not explain variation in performance of thermoregulatory behaviors

For birds exposed to hot temperatures, we tested whether variation in morphology explains variation in time invested in thermoregulatory behaviors using linear models. We did not find support for our predictions. Birds of greater mass did not pant more ($F_{1,4}$ =0.74, p-value=0.43). Birds with greater beak surface area ($F_{1,4}$=1.53, p-value=0.284) or larger tarsi $F_{1,4}$=1.01, p-value=0.372) also did not pant more. For birds exposed to cold temperatures, we asked whether those of greater mass spent less time with their feathers extended, but did not find a correlation ($F_{1,4}$=0.08, p=0.79).

PART 4: DISCUSSION

The main purpose of this investigation was to characterize the thermoregulatory and non-thermoregulatory behavior of zebra finches at temperatures below, within, and above their thermoneutral zone. In some ways, the investigation was successful. For instance, despite the relatively small sample size we were able to determine that temperature manipulations of as little as 3°C outside the zebra finch thermoneutral zone can cause significant changes in thermoregulatory behavior. Postural states were significantly different between the relatively cold (27°C), thermoneutral (35°C), and relatively hot (43°C) temperature treatment groups, with those birds in the colder group spending significantly more time in a piloerect state, and those birds in the hotter group spending significantly more time panting, as compared to birds held within their thermoneutral zone. We were also able to compare behavior within individuals after two hours of temperature exposure as compared to four or more hours to determine that a two-hour exposure is sufficient to elicit thermoregulatory behaviors, such as panting or piloerection. Yet in other ways, the investigation was unsuccessful in that we were unable to fully characterize behavioral differences between and within treatments. For instance, while we found significant differences in behavioral states between treatment groups, with a sample size of 6 we were unable to assess individual variation in thermoregulatory behavior, or to determine whether individual variation was correlated with any aspect of morphology, as predicted. In addition, with our limited sample size we were unable to determine whether temperature affected non-thermoregulatory behaviors such as eating, drinking, grooming, and activity. Although we found certain trends for differences in these behaviors among temperature treatments, a larger sample size is needed for sufficient power to assess the significance of those differences. This is a particularly important question, as there may be trade-offs between thermoregulatory behaviors and fitness-related behaviors, such as foraging (du Plessis et al. 2012; Gardner et al. 2016).

Although limited by a small sample size, we were able to compare our estimates of time spent dissipating heat with data from wild observations. Free-living zebra finches spend an average of 20% of their time panting at 27°C, 60% of their time panting at 35°C, and 90% of their time panting at 43°C (Funghi et al. 2019). In comparison, we found that captive birds spend 0% of their time panting at 27°C, 6% at 35°C and 75% at 43°C. Although we also found a steep increase in panting behavior as temperatures rose, at cooler temperatures we found that lab-housed zebra finches spend much less time panting than free-living ones. One possible explanation is that free-living birds were exerting themselves significantly more, even in cooler temperatures, and thus had more exercise-induced body heat to shed. Current evidence suggests that wild and domesticated zebra finches do not differ in their thermoneutral zone or thermal physiology (Beaulieu 2016), but whether they differ in behavior in the absence of activity differences is unknown, and the cause of this discrepancy should be studied further. Comparing time spent in warming thermoregulatory behaviors (i.e. piloerection) with data from the wild is not possible, as to our knowledge there are no studies quantifying these behaviors in wild populations of zebra finches.

Because we did not find significant differences between temperature treatment groups in other behaviors, such as eating or movement, it is difficult to know whether our results are consistent with observations of free-living or domesticated birds. In poultry, higher temperatures result in a reduced appetite and movement (Pilo et al. 1985; Daghir 2008). This was consistent with trends in our data, although interestingly movement did not decline with temperature, but rather peaked at 35°C. Still, further testing should reveal whether this pattern is consistent with a larger sample size. While grooming has not, to our knowledge, been studied in the context of temperature, it would be interesting to investigate further whether the trends found in this study (more grooming behaviors below and above the thermoneutral zone) hold true with larger sample sizes. One possible explanation for this trend is that temperatures outside of the thermoneutral zone cause more discomfort for birds, or that they are using preen-oil from their uropygial glands (reviewed in Moreno-Rueda 2017) to modulate temperature. This latter possibility has been relatively unexplored in preen-oil literature: while one study found that preen-oil reduced heat loss in mallard ducklings (*Anas platyrhychos;* Bakken et al. 2006), the effect of temperature on grooming and preening behavior has remained largely understudied. As with other behavioral events, we did not find significant differences in drinking behavior in the hot group as compared to the cooler groups, as we expected to find. This was an interesting finding because unlike other behaviors, there was not even a trend of drinking more in the hot group, despite significantly increased panting behavior. While more data is needed to confirm that heat does not dramatically increase drinking behavior in zebra finches, this finding may be a reflection of the arid conditions to which zebra finches are adapted (Calder 1964; Zann 1996) and their efficiency in retaining water. Although we predicted that zebra finches would drink water if available to them, Calder (1964) found that zebra finches on a water restriction were just as capable of evaporative cooling as non-restricted birds up to 43.5°C. Thus our treatment may not have been hot or long enough to require significantly more water resources, given the remarkable water-conserving abilities of zebra finches.

While the purpose of this study was to investigate heat-dissipating behaviors and compare them to behaviors at cooler temperatures, our most immediately applicable findings may have been on the heat-trapping behavior observed in the cold treatment group. In a review on common housing conditions and native climatological conditions of zebra finches, Beaulieu (2016) noted that the vast majority of zebra finches used for research purposes are housed at temperatures below their thermoneutral zones and inconsistent with natural light cycles and humidity levels. Even when temperatures are noted in experimental design, lower temperatures are often considered the less stressful condition because zebra finches have supposedly acclimated to these conditions (e.g. Hoffman et al. 2018). Yet despite being housed at these typical housing temperatures of 24 – 25°C for two weeks before experimentation, we found that zebra finches in the cold (27°C) group spent more than half their time, on average, in a piloerect state. This indicates that zebra finches are actively thermoregulating even at temperatures 3°C *above* typical housing temperatures, and even after a time period in which they are typically assumed to have acclimated to cooler temperatures. While more research is needed to determine whether being housed below the thermoneutral zone affects cognitive behaviors that are commonly studied in zebra finches (Healy et al. 2010), it should certainly be noted that such zebra finches are frequently being studied in what may be a stressful state.

The overarching goal of this research was to understand how individuals vary in their response to heat, what the species limitations are in response to heat, and thus how populations of animals will adjust to rising temperatures and more extreme weather. Behavioral characterization such as this is one important step towards that end, by providing a framework with which to compare future investigations, and with a larger sample size we will be able to more accurately describe variation in behavioral response at different temperatures. The next step will be to integrate information about behavioral variation with variation in morphology, to determine whether rising temperatures may favor certain morphological traits, and to integrate behavioral information with information about variation in the cellular response to heat (Evgen'ev et al. 2014), to determine whether rising temperatures may select for different responses at the cellular level. With this more complete picture, we will be better positioned to estimate how populations will or will not be able to adapt to a changing climate.

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APPENDICES

Appendix A

Ethogram used for behavioral quantification

States:

- **Panting**: beak is open and bird is breathing with some rapidity. Typically visible via open beak and chest movements. Birds typically open and close beak through a panting session. A closed beak indicates a cessation in panting when it has been closed for five counts (approx. 3 seconds). If the beak is closed longer than five counts, it typically stays closed for some time until panting resumes again, whereas shorter times frequently lead to resumed panting.
- **Neutral Posture**: standing or moving without any noticable postural changes.
- **Out of View**: not in view of camera, either due to camera positioning or video freeze.
- **Piloerection**: anytime the bird remains for longer than one count in a puffed position, typically visible via extended feathers and a larger-looking body size. Different than a fluff (see below).
- **Time aloft:** measured as the proportion of time the bird is either on a perch, the side of the cage, or on the thermal probe. In other words, time not spent on the floor, the food dish, or the water dispenser.

Events:

- **Eat:** defined as a single head-bob down to the food (typically they get one seed, raise their head, eat it, then bob down again for another)
- **Drink:** defined as one head bob into the water dish
- **Groom:** defined as any time the bird twists its head around to touch beak to feathers or preen-oil gland
- **Fluff**: defined as any time the bird erects all feathers at once and then settles them within one second (distinct from piloerection, in which they remain erect, typically to a lesser degree)
- **Move:** anytime both feet have left the ground. This can either include a movement from perch to perch, perch to side, a lateral hop on the perch, a twist (facing forward to facing back) on the perch, a hop on the ground, or any movement from perch to ground to food dish, etc.

Table 1: Behavior differences between temperature treatment groups. Results reported for one-way ANOVAs and post-hoc Tukey's tests. Significant differences reported in bold.

		Mean (number of events or percent of time)			One-Way ANOVA		Tukey's HSD p-values		
	Behavior	Cold	TNZ	Hot	F-value	p-value	Cold-TNZ	TNZ-Hot	Hot-Cold
States	Panting	0%	5.9%	75.2 %	27.087	**0.0000105**	0.862	**0.0000017**	**0.0000003**
	Piloerection	53.0 %	5.2%	0%	21.80	**0.0000364**	**0.00206**	0.826	**0.0000693**
	Neutral Posture	44.1 %	88.5 %	24.8 %	11.53	**0.000925**	**0.0136**	**0.000806**	0.356
	Time Aloft	56.3 %	45.6 %	10.8 %	3.34	0.063	0.833	0.175	0.0633
Events	Eat	14.0	10.2	5.0	2.204	0.145	0.654	0.471	0.125
	Drink	3.7	1	2.7	1.1	0.37	0.347	0.649	0.853
	Fluff	5	3.7	1.2	4.36	0.0322	0.581	0.174	0.027
	Groom	10.8	3.7	13.2	1.862	0.190	0.367	0.187	0.893
	Move	162.5	195.2	82.3	1.43	0.270	0.885	0.259	0.489

Appendix B

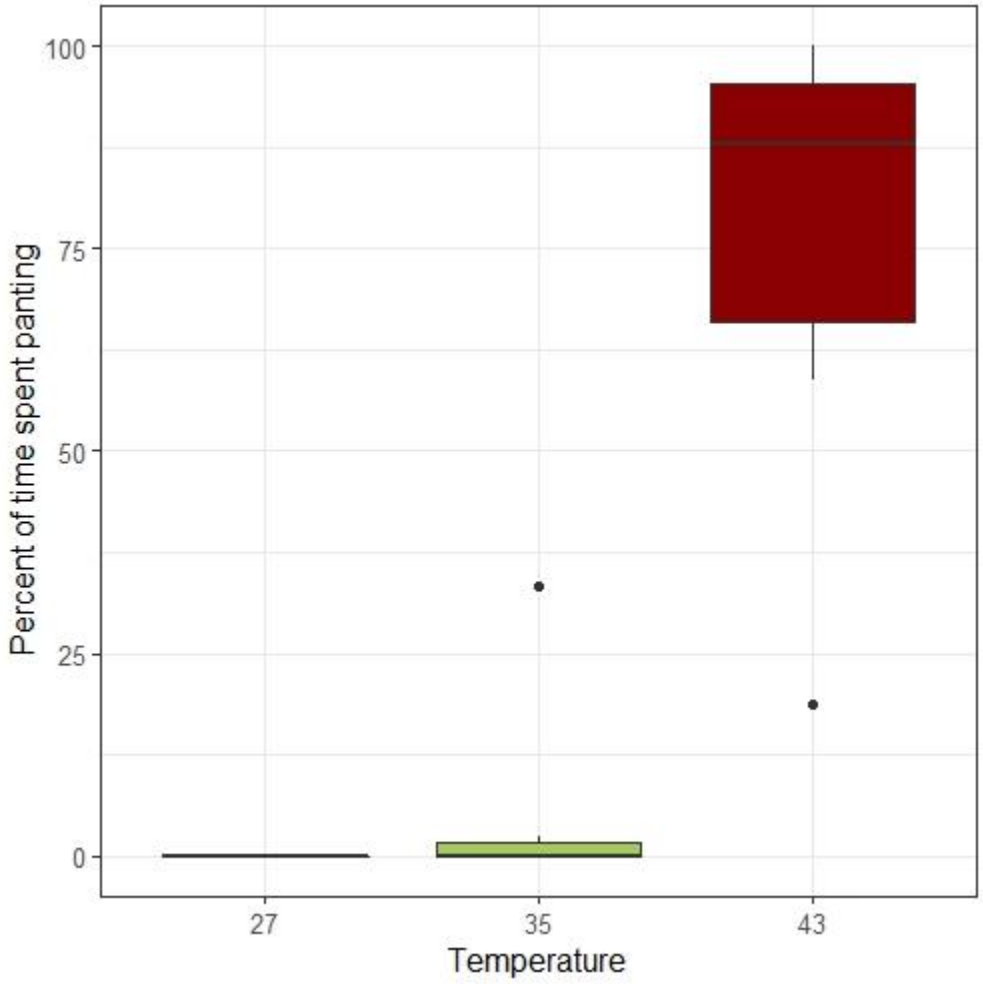

Figure 1: Percent of time spent panting at 27°C, 35°C, and 43°C (N=6)

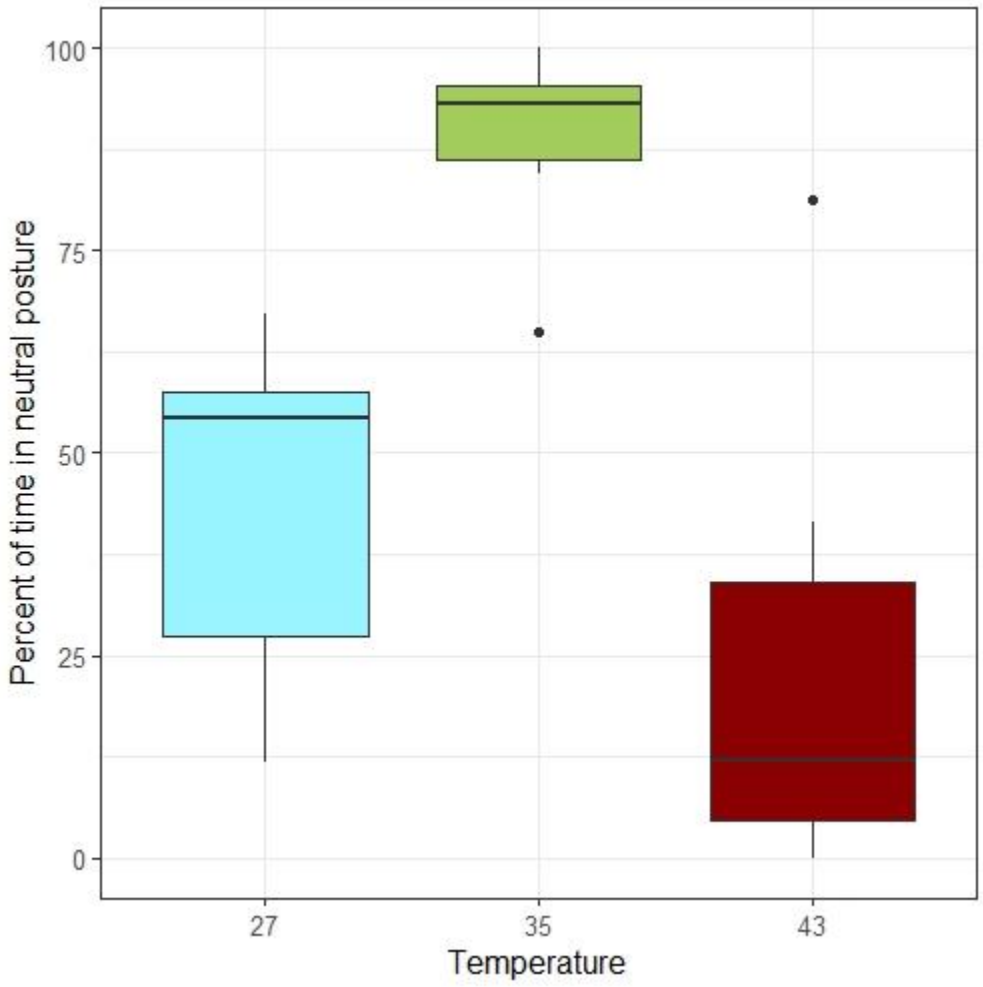

Figure 2: Percent of time spent in a neutral position at 27°C, 35°C, and 43°C (N=6)

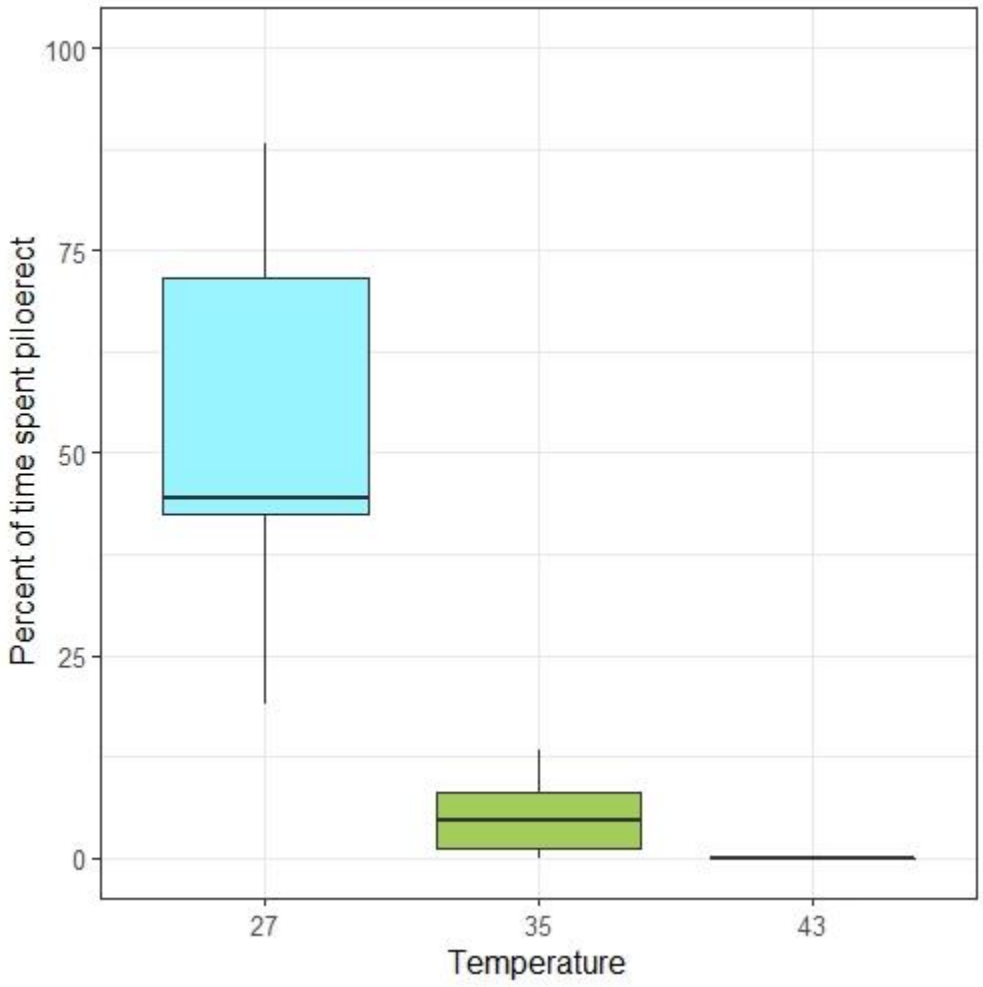

Figure 3: Percent of time spent piloerect at 27°C, 35°C, and 43°C (N=6)

Appendix C

Table 2: Behavior differences between temperature treatment groups during the second time point, immediately before cessation of treatment. Results reported for one-way ANOVAs and post-hoc Tukey's tests. Significant differences reported in bold.

		Mean (number of events or percent of time)			One-Way ANOVA		Tukey's HSD p-values		
	Behavior	Cold	TNZ	Hot	F-value	p-value	Cold-TNZ	TNZ-Hot	Hot-Cold
States	Panting	0%	5.1%	81.1 %	116.9	**7.1×10^{-10}**	0.676	**0.00000**	**0.00000**
	Piloerection	54.9%	4.9%	0%	11.90	**0.000807**	**0.00305**	0.919	**0.00140**
	Neutral Posture	43.3%	87.0 %	17.8 %	14.88	**0.000275**	**0.0103**	**0.000207**	0.149
	Time Aloft	43.3%	38.9 %	26.1 %	1.03	0.380	0.691	0.821	0.351
Events	Eat	16.3	9.8	9	0.58	0.571	0.665	0.994	0.597
	Drink	3.3	1.2	1.3	0.93	0.418	0.459	0.995	0.512
	Fluff	4.3	3.8	3.7	0.069	0.934	0.961	0.996	0.933
	Groom	5.2	6.8	18	1.87	0.189	0.971	0.299	0.211
	Move	149	126.3	92.3	0.24	0.788	0.959	0.910	0.772

Vita

Clara Howell is originally from Morrison, Colorado. From 2013 to 2017 she attended Tulane University in New Orleans and obtained a Bachelor's of Science in Neuroscience and a Bachelor's of Arts in English. Her undergraduate research focused on the role of cognitive ability in zebra finch mate choice. While studying both at Tulane University and at the University of Tennessee, Knoxville, she was a member of Dr. Elizabeth Derryberry's behavioral ecology lab. She plans to continue her studies with a Ph.D. in Biology from Duke University.