

Organismal effects of heat in a fixed ecological niche: implications on the role of behavioral buffering in our changing world

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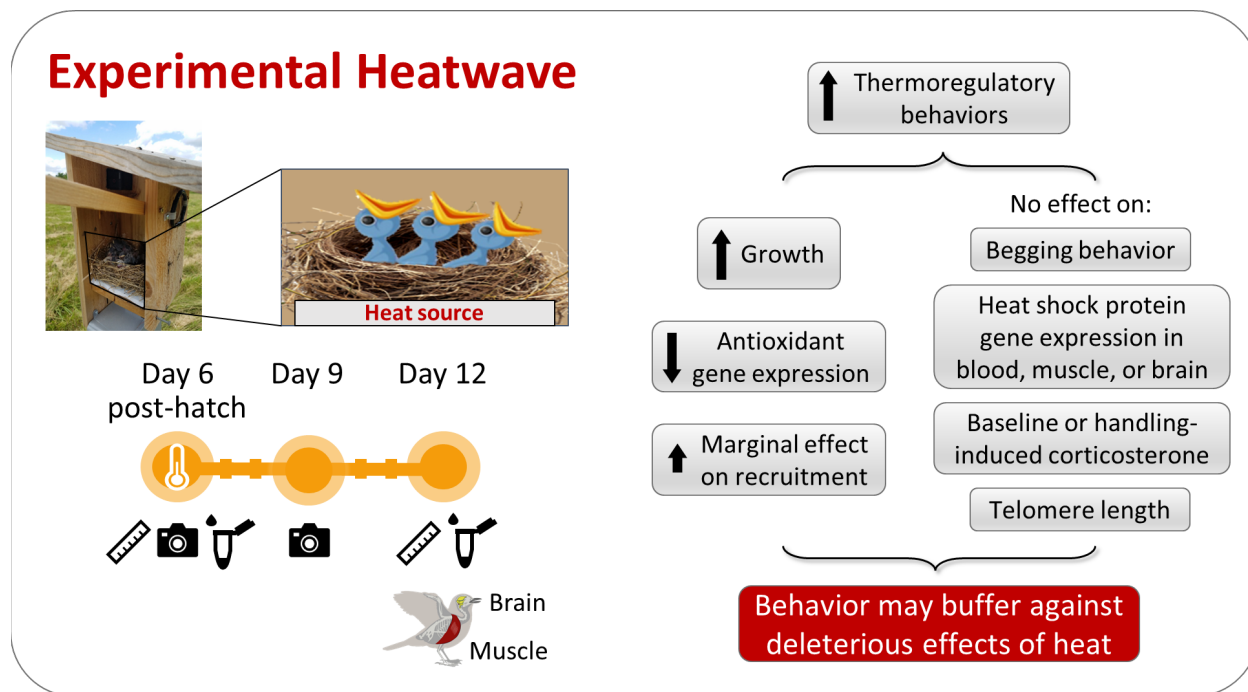
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Abstract

Increasingly frequent and intense heatwaves generate new challenges for many organisms. Our understanding of the ecological predictors of thermal vulnerability is improving, yet, at least in endotherms, we are still only beginning to understand one critical component of predicting resilience: exactly how do wild animals cope with sub-lethal heat? In wild endotherms, most prior work focuses on one or a few traits, leaving uncertainty about organismal consequences of heatwaves. Here, we experimentally generated a 2.8°C heatwave for free-living nestling tree swallows (*Tachycineta bicolor*). Over a week-long period coinciding with the peak of post-natal growth, we quantified a suite of traits to test the hypotheses that (a) behavioral or (b) physiological responses may be sufficient for coping with inescapable heat. Heat-exposed nestlings increased panting and decreased huddling, but treatment effects on panting dissipated over time, even though heat-induced temperatures remained elevated. Physiologically, we found no effects of heat on: gene expression of three heat shock proteins in blood, muscle, and three brain regions; secretion of circulating corticosterone at baseline or in response to

handling; and telomere length. Moreover, heat had a positive effect on growth and a marginal, but not significant, positive effect on subsequent recruitment. These results suggest that nestlings were generally buffered from deleterious effects of heat, with one exception: heat-exposed nestlings exhibited lower gene expression for superoxide dismutase, a key antioxidant defense. Despite this one apparent cost, our thorough organismal investigation indicates general resilience to a heatwave that may, in part, stem from behavioral buffering and acclimation. Our approach provides a mechanistic framework that we hope will improve understanding of species persistence in the face of climate change.

Graphical Abstract:



Keywords

Heat Tolerance, Climate Change, Brain, Behavior, Juvenile, Avian, Stress, Thermal Adaptation, Resilience, Acclimation

Introduction

As heatwaves become more frequent and intense (Fischer et al. 2021), many species face heat-challenges. If we are to predict species resilience, we need a deeper understanding of the mechanisms by which wild animals cope with sub-lethal heat. Behavioral plasticity is one way organisms can cope with changing environments (West-Eberhard 2003), including heat (Huey et al. 2003; Huey et al. 2012; Muñoz 2022). Both plants (van Zanten et al. 2021) and animals (Verzuh et al. 2021) move away from heat, and animals may change activity patterns to reduce physiological costs of heat (Bourne et al. 2021), but these options may not be possible in a fixed ecological niche, including for sessile organisms (e.g., Pandolfi et al. 2011) and altricial young (e.g., Eastwood et al. 2022; Larson et al. 2015). To cope with unavoidable heat, animals use postural changes (du Plessis et al. 2012) and evaporative cooling (e.g. panting, bathing) (Loughran & Wolf 2020; Oswald et al. 2008). However, they may eventually be pushed beyond their thermoneutral zone - a range of environmental temperatures in which animals spend minimal metabolic energy thermoregulating (Angilletta 2009). At some point, elevated temperatures may require additional coping mechanisms.

Physiological changes are also beneficial for coping with challenging stimuli. For instance, elevated internal body temperatures can improve water economy during heat (McKechnie & Wolf 2019). Glucocorticoid hormones can mobilize resources to provide energy for handling or recovering from stress (Mentesana & Hau 2022; Romero et al. 2009), including stress caused by heat (Kang & Shim 2021). Heat can also induce oxidative damage (Cheng et al. 2018), but antioxidants can be upregulated to combat this issue (Cheng et al. 2018; Jiang et al. 2015). Animals can also upregulate heat shock proteins (HSPs), which serve to minimize and repair protein dysfunction (Feder & Hofmann 1999; Lindquist & Craig 1988). Across diverse taxonomic groups, HSPs have been elevated in response to heat (Feder et al. 1999; Hoffmann et al. 2003; Lipshutz et al. 2022). Although these physiological mechanisms are adaptive in the short term, they also may direct energy away from other critical functions, leading to short- or long-term deficits. Further, if stress is chronic or extreme, oxidative damage can accumulate (Fontella et al. 2005), leading to accelerated ageing (Chatelain et al. 2020) and reduced longevity (Sánchez-Hidalgo et al. 2016).

Juvenile animals may be particularly vulnerable to heat. Heat during early life can negatively affect development, growth, and survival (Silva et al. 2021; Ujszegi et al. 2022), with repercussions for later reproductive success (Sales et al. 2021), though mild heat may have benefits for growth (Kingsolver et al. 2020). In birds, past research is mixed as to how heat affects developing young (Andreasson et al. 2018; Andrew et al. 2017; Corregidor-Castro & Jones 2021; Dawson et al. 2005; Hsu et al. 2020; Rodriguez & Barba 2016; Ton et al. 2021). Each of these studies has generated important insights, but they generally focus on one or a few traits, leaving uncertainty as to whether heatwaves have net positive or negative effects, given the potential for trade-offs among organismal traits (Stearns 1992). For instance, body mass in young birds predicts their early life survival and later fecundity (Gebhardt-Henrich & Richner 1998), but these benefits are countered by other viability costs, e.g., related to growing too fast or the need for a protracted developmental window (Blanckenhorn 2000). Organismal perspectives are needed so that we can interpret the valence of these heat effects.

We experimentally simulated a week-long heatwave of ~2.8°C inside the nesting cavity of free-living nestling tree swallows (*Tachycineta bicolor*). We quantified thermoregulatory behaviors (i.e., panting and huddling) and several physiological mechanisms, including: (i) gene expression of heat shock proteins in blood, skeletal muscle, and three brain regions, as well as (ii) glucocorticoid secretion at baseline and in response to a restraint stress, and (iii) gene expression for superoxide dismutase, a key regulatory enzyme in counteracting oxidative damage (reviewed in Wang et al. 2018). We also quantified performance-related consequences including: (i) changes in nestling begging behavior, (ii)

growth rate, (iii) relative telomere length, which is a proxy of future longevity (Wilbourn et al. 2018), (iv) likelihood to fledge, and (v) likelihood to recruit to the breeding population. Using these data, we tested the hypothesis that behavior is the first line of defense in buffering animals from the deleterious effects of heat (sensu Huey et al. 2003; Huey et al. 2012; Muñoz 2022). Based on this hypothesis, heat should induce thermoregulatory behaviors, with limited effects on physiological traits and null or positive effects on performance. These analyses, which assess both immediate and enduring effects, will lend insight into mechanisms of thermal tolerance in our changing world.

Methods

Study Species and Location: Tree swallows are cavity-nesting birds that nest in human-made nest boxes. Our experiment focused on nestlings confined to this thermal environment near Bloomington, Indiana USA (39.1653° N, 86.5264° W), where we monitor over 150 tree swallow nests each year. Focal nests were located at one of two wet, meadowy sites separated by 14.2 km. In this species, mothers alone incubate eggs and brood nestlings frequently until they develop homeothermic capabilities (Winkler et al. 2020). Nestlings begin thermoregulating around day 6 post-hatch (D6) (Marsh 1980), while undergoing a period of rapid growth until they reach asymptotic, adult-like mass around D12 (McCarty 2001). Both parents provision nestlings, which fledge around D21 (Marsh 1980); hatch day is denoted as D1. Broods are typically 4 to 7 nestlings (Winkler et al. 2020), with an average of 4.7 nestlings in this study (range: 2-6).

Simulated Heatwave: Heatwaves are generally defined as a period of two to several days with temperatures above what is normally expected (Heo et al. 2019; Xu et al. 2016). Our experiment sought to mirror such an event, rather than a set target temperature per se. Using an air-activated warmer (Uniheat 72h) placed directly under the nest, we elevated nest temperatures starting at D6 post-hatch between 8:00 and 10:00, continuing until D12 post-hatch. Warmers contain a mixture of charcoal, iron powder, vermiculite, salt, sawdust, and moisture. When the warmer packaging is opened, exposure to air leads to heating via oxidation of iron powder, yielding a heat challenge above naturally occurring temperatures. Uniheat warmers were rated for 72h, but pilot work showed that temperatures waned after ~24h; therefore, we replaced warmers daily. In control nests, cooled warmers were used in an identical fashion to control for non-thermal disturbances of the experimental design. To reduce among-nest variation in treatment effectiveness, we standardized the amount of nesting material between the warmers and the nestlings; for nests >7cm tall, we removed grass from the bottom of the nest, and for nests ≤2.5cm, we added thin posterboard between the bottom of the nest and the warmer. Treatments were balanced by date, brood size, and age of the mother. The experiment occurred between May and July 2019. Two heated nests were excluded for failures unrelated to the experiment, leaving n=17 heated nests, n=21 control nests, with n = 80 heat nestlings, n = 102 control nestlings.

To habituate birds to foreign objects, a dummy camera and iButton temperature logger were placed in the nest on D4 post-hatch (i.e., 2 days before treatments began). Each iButton was fastened into the nest, facing up, recording temperature every 20 min. Ambient data came from NOAA hourly dry bulb temperature from a nearby weather station (ID: WBAN:03893; avg. 14.3km from our study areas). We collapsed iButton reads into hourly averages to mirror NOAA data. For each nest, we calculated the hourly difference between nest and ambient temperatures as well as the mean nest temperatures across the duration of experiment (D6-12). Two nests have missing or excluded temperature data: (1) one nest (control) experienced an iButton technology failure, and (2) one iButton (heat) recorded uncharacteristically high temperatures coinciding with the D11 warmer swap, suggesting the iButton was dislodged from its standardized position. For this latter nest, we only included nest temperatures from D6-D10 in the final dataset.

Because iButtons faced upwards during the experimental heatwave, they did not directly measure heat coming up from the heat source below, which may lead to under-estimation of the treatment effect. Therefore, we conducted a post-hoc experiment to compare iButtons facing up versus down (elaborated in *SI§a*).

Nestling Behavior: We measured behavior on the first and third day of the experiment. We mounted cameras (GoPro Hero Session 4) in each box during warmer placement. Cameras recorded for two hours, and we used only the second hour of data to allow recovery from human disturbance. We scored videos in JWatcher (version 1.0; Blumstein & Daniel 2007). First, we measured the total number of minutes any panting was observed. Second, we measured the number of nestlings huddling inside (versus outside) the nest cup. Finally, we recorded the number of nestlings that begged per feeding, and for each nestling, we scored begging intensity using a postural scale from 0 to 4 (adapted from Pilz et al. 2004). See details in *SI§b* and *Figure S1*.

Parental Visitation: To account for potential indirect effects of heat on parents, we measured parental visitation rate using radio-frequency identification (RFID) boards, as a proxy for provisioning rate (Lendvai et al. 2015). In our population, all mothers and most fathers were banded with a plastic leg band containing a PIT tag. Due to logistical constraints, we only measured visitation at the first 26 of 38 nests. Samples sizes per treatment are lower than this because of malfunctions in some PIT tags (see *Table 1*). See *SI§c* for details.

Blood Sampling: We collected nestling blood between 12:00 – 16:00 on D6, D9, and D12. On D6 and D9 we sampled all nestlings, and on D12 we sampled 1-3 nestlings per nest depending on brood size, avoiding runts but otherwise choosing at random. D9 and D12 samples were taken from the alar vein, but D6 samples were collected from the medial metatarsal vein because the wings were insufficiently developed at this age. For gene expression analyses, we collected 20-50µl on D6, D9, and D12. For baseline and handling-induced corticosterone (Cort) secretion analyses, we collected two 20µl samples on D12. Following best practices (Gaunt et al. 2010), we did not collect >2% body mass summed across samples, and we did not exceed 1% in any one sampling. In the field, blood samples were placed on wet ice (i.e., for hormone analyses) or dry ice (i.e., for gene expression). In the lab, we stored plasma at -20°C and other tissues at -80°C.

On D6, we timed our sampling to 4h after the onset of heat because previous work in poultry shows robust changes in HSP gene expression within 4h of heat (Tu et al. 2016; Xie et al. 2014). On D9 and D12, samples were collected approximately 72h and 144h after the D6 bleed, respectively.

On D12 only, we collected blood for Cort and gene expression analyses, modifying our bleeding protocol as follows: First, we collected a baseline sample within 3 min of opening the nest box. As a continuation of this bleed, we took a second tube for gene expression analysis (completed on average, 2.56 min ± 0.17 after our arrival to the nest). Finally, 30 min after disturbing the nest box, we collected a final sample to measure handling-induced (or elevated) Cort (Romero & Reed 2005). Latency to complete the gene expression sample was not related to handling induced Cort (LMM with the random effect of nest ID: D12 gene expression bleed end time: $F_{1,42.17} = 0.99$, $p = 0.33$). Later that day, we centrifuged samples intended for Cort assays, reserving plasma via Hamilton syringe. The remaining red blood cells were used for DNA-based telomere length analyses, described below.

Morphology: On D6, each nestling was given a unique pattern of nail clipping (keratin nail tip blunted), for individual identification. On D6, D9, and D12, we measured mass to the nearest 0.1g using a digital scale (HH120D, Ohaus, USA). On D12, we measured wing length to the nearest 0.5 mm using a stoppered wing ruler, and banded nestlings with a numbered USGS band.

Terminal Collection: At the end of the experiment (D12), we euthanized one nestling/nest in 25 nests, and snap-froze additional tissues on dry ice. We selected the median mass chick for these analyses because body mass can impact heat responses (Choy et al. 2021). We later micro-dissected brains following (Bentz et al. 2019) for region-specific analysis. We focused on the hypothalamus (HYPO), hippocampus (HPC), and ventromedial telencephalon (VmT, which includes the avian medial amygdala or nucleus taeniae) because these regions contain social and cognitive centers in the brain (Goodson 2005; O’Connell & Hofmann 2012). In addition, HYPO mediates homeostatic temperature regulation (Murugesan et al. 2017). HPC is involved in navigation and spatial memory (Bingman et al. 2003; Pravosudov et al. 2006), traits that likely affect successful migration (Mikami 1986). Our final tissue was the pectoralis, the major flight muscle, which is critical for successful fledging. In a previous study, we found that these tissues express HSP mRNA in unmanipulated, wild adult tree swallows, with lower levels in muscle compared to brain (Woodruff et al. 2022). In addition, some brain regions show latitudinal variation in HSP gene expression, suggesting that HSPs may be related to thermal regimes (Woodruff et al. 2022).

Quantitative PCR: We extracted RNA using Trizol, and we converted RNA to cDNA using Superscript III (elaborated in *S1d*). cDNA was used in quantitative real-time PCR (qPCR) to measure mRNA abundance of SOD in blood as well as three HSPs (HSP90AA1, HSP90B1, and HSPA2) in blood, brain, and pectoral muscle. We focused on these HSPs because they have been robustly linked to heat tolerance (Wan et al. 2017; Wang et al. 2015). We ran qPCR reactions in triplicate on 384-well plates, alongside no template controls (NTCs), in a QuantStudio 5 thermocycler (Thermo Fisher Scientific, Massachusetts, USA) using PerfeCta SYBR Green FastMix with low ROX (Quanta Biosciences, Maryland, USA). See *S1d* for details on qPCR reactions and thermal profiles. We calculated mRNA abundance with the comparative Ct method ($2^{-\Delta C_t}$): fold change in expression for each gene of interest, normalized to an internal reference gene. We used MRPS25 as a reference gene for blood and pectoral data, and the geometric mean of HMBS and PPIA for brain data because these genes did not significantly differ in expression between treatments (MRPS25: $F \leq 0.65$, $p \geq 0.43$; HMBS and PPIA: $F \leq 1.3$, $p \geq 0.28$). Primer details are reported in *Table S1*. Each qPCR plate included intra- and inter-plate control samples (a cDNA pool derived from tree swallow RNA). Intra-plate CV was $0.86 \pm 0.56\%$ and inter-plate was CV 1.78%.

Quantifying Plasma Corticosterone: Using an enzyme immunoassay kit (Cayman #501320), we quantified plasma corticosterone as in (Virgin & Rosvall 2018); elaborated in *S1e*. We selected two middle-mass nestlings from each nest for this analysis – the same for which we measured blood HSP gene expression. 12 of 142 samples had unexpectedly high variation among duplicates (CV >25%), so we omitted these data. We then calculated intra-plate variation by averaging across all sample and pool CVs for each of 5 plates. Intra-plate CV was $13.40 \pm 3.62\%$. Inter-plate CV was 22.66%, and plates were balanced by treatment.

Quantification of Telomere Length: We quantified relative telomere length using DNA extracted from D12 red blood cells following Wolf et al. (2021). We used primers *telc* and *telg* (Cawthon 2009) to quantify telomere length relative to the single copy gene GAPDH; see *Table S1* for details. We ran samples in triplicate and used mean values to calculate the ratio (T/S) of telomere repeat copy number (T) to a single gene copy number (S) for each sample using the formula: $2^{-\Delta\Delta C_t}$ ($\Delta\Delta C_t = (C_t^{\text{telomere}} - C_t^{\text{GAPDH}})_{\text{sample}} - (C_t^{\text{telomere}} - C_t^{\text{GAPDH}})_{\text{reference}}$). All plates contained a reference sample of pooled tree swallow DNA, which yielded intra-plate CVs of 0.42% for GAPDH and 0.83% for telomeres. Using 28 samples run across two plates, the inter-plate repeatability of the T/S ratio was high (intraclass correlation coefficient: 0.86; 95% confidence interval: 0.73–0.93). Plates were balanced by treatment, hatch date, and brood size.

Survival and Recruitment: At the end of the experiment (D12) and after fledging, we inspected the nest for dead nestlings. We also devoted considerable effort during the next two years to identify and

capture subjects from this experiment that recruited to the population as adults, identified by their USGS band (elaborated in SI§g). This approach provides a good estimate of recruitment (established methods for this species e.g., Lombardo et al. 2020) because typical natal dispersal distances are 8.38 km for females and 2.44 km for males (Winkler et al. 2005), and our extensive study population spans 36.4 km. Typical recruitment rates for this species are 5-10% (Lombardo et al. 2020; Shutler et al. 2006; Wolf et al. 2022).

Statistical Analysis: Statistical analyses were performed with JMP v14 (SAS Institute, Cary, NC) and R (RStudio Version 1.3.959). Sample sizes are summarized in *Table 1*.

Temperature: To quantify our experimental heatwave, we tested for effects of treatment on nest temperature elevation above ambient because this metric accounts for ambient daily variation. Temperature elevation was calculated by subtracting the hourly mean nest temperature from its corresponding hourly ambient value. Temperatures in the wild are expected to vary non-linearly with hour and day, so we fit a generalized additive model (GAM) that allows for non-linear data with restricted maximum likelihood (REML) using the *mgcv* R package (Wood 2020). Main effects were treatment, brood size, a smoothed (i.e., non-linear) term for experiment hour, and a smoothed term for the interaction between treatment and hour. Experiment hour 1 began when the warmer was placed in the nest box on D6 and continued until the D12 sample collections ($147.7\text{h} \pm 1.4$). Smoothed variables use a penalized thin plate regression spline to correct for overfitting (Wood 2006). Our model assumed Gaussian distribution with a log link function, and included the random effect of nest ID. We inspected model fit using *gam.check* in the *mgcv* package.

Treatment effects on other variables: Next, we used a model comparison framework based on Akaike Information Criterion to identify the single model that best fit our data (lowest AICc score) (Burnham & Anderson 2002); or, in cases where multiple models had ΔAICc values ($\text{AICc}_i - \text{AICc}_{\text{best model}} \leq 2$), we considered them equally fit and averaged them using MuMIn (Barton 2019). We used the dredge function (Barton 2019) to create model sets that evaluated and controlled for potentially relevant fixed effects. For instance, ambient temperature could affect temperatures experienced by nestlings, so we include mean ambient temperature across all hours of the experiment. Likewise, brood size could impact conductive heat transfer among siblings, so we include the number of nestlings in the brood. We included treatment in every model to provide a statistical test of our core research question. Therefore, our most simplistic model was our ‘focal’ model, which included only treatment and the intercept. We included the random effect of nest ID in all models that included multiple measures per nest. We also ensured that variables were not multicollinear (ensured that variable inflation factors were <3 , Fox & Weisberg 2018). All models followed best practices of at least one parameter per 10 observations. Data and model residuals were visually inspected for normality. Unless otherwise stated, models assumed gaussian distribution. We Log_2 transformed relative gene expression values when it improved normality. We Log_{10} transformed Cort data for analysis, though figures plot untransformed data to facilitate biological interpretation.

In the text, we present results from the model(s) that best fit our data (i.e., the lowest ΔAICc , or equally fit, ΔAICc), including variance explained by fixed (R^2 marginal) and random effects (R^2 conditional) when applicable. *Table S5* reports these models alongside the focal model. We report effect sizes via beta estimates (β), standard error, test statistics, and p-values. Treatment effect β s are relative to controls. For averaged models, we report full model-averaged coefficients including adjusted standard error and z values (full and conditional model-averaged coefficients in *Table S6*).

Behavior: For each nestling behavior – duration panting, number of nestlings huddling, mean number of nestlings begging, and mean begging intensity – model sets included our focal model, plus models with

treatment and every combination of timepoint (D6 or D9), the interaction between timepoint and treatment, ambient temperature, and brood size. Because total duration panting was zero-inflated, we used a negative binomial regression. To examine differences in heat effects across timepoints, we performed post-hoc least square mean comparisons for significant interactions.

We also tested for potential confounding effects of heat on mean daily parental visitation rate. We tested each parent separately because heat may affect mothers and fathers differently (Perez et al. 2008). Model sets included our focal model, plus every combination of ambient temperature and brood size.

Morphology: To assess heat effects on growth, our dependent variable was the percent change in mass from D6 to D12. For simplicity, we did not use D9 mass data, instead focusing on growth occurring from the beginning (D6) to the end (D12) of heat. In addition to our focal model, sets also included D6 mass (i.e., starting mass) and the interaction between D6 mass and treatment. Analysis included $n = 171$ nestlings for which we had all predictor variables. We also evaluated effects of heat on D12 wing length. Model sets included our focal model plus every combination of ambient temperature and brood size.

HSP gene expression: The three HSPs of interest were positively correlated with each other (range of Pearson $r = 0.33-0.68$, see *Table S2*), so we collapsed them using Principal Components Analyses (PCA; loadings in *Table S3*). We performed separate PCAs for each tissue because we were interested in tissue-specific heat effects, and some tissues differed in expression of the reference gene, thereby preventing direct comparisons of mRNA abundance. Similarly for blood, we performed separate PCAs at D6 and at D12 because the reference gene changed in expression from D6 to D12. Due to limited resources, we opted to exclude D9 gene expression data from this project, though we note that a pilot analysis mirrors the D6 and D12 results below (see *S15f*, including *Table S4*). We therefore focused on timepoint- and tissue-specific comparisons between treatment and control at D6 or D12. For each tissue, we used the first PC, which explained 45.2-85.5% of the variation in HSP gene expression (eigenvalues ≥ 1.4 ; *Table S3*). Next, we evaluated model sets for each PC1 that included our focal model and every combination of ambient temperature and brood size.

Corticosterone: Model sets included the focal model with a random effect of nest ID, plus every combination of ambient temperature and brood size. We analyzed baseline and handling-induced Cort separately because evidence suggests these endocrine traits are regulated and can evolve independently (Vitousek et al. 2019).

SOD gene expression: Model sets predicting D12 SOD gene expression included our focal model, plus every combination of ambient temperature and brood size. Analysis included 1 nestling per nest.

Telomere length: Model sets predicting relative telomere length on D12 included our focal model, plus every combination of ambient temperature and brood size, while controlling for the random effect of nest ID.

Likelihood of survival and recruitment: First, to confirm that the treatment was sublethal, we performed a logistic regression using a generalized linear mixed model with a binomial distribution to look for effects of treatment on the likelihood of nestlings to survive to D12, while controlling for the random effect of nest ID. Data stem from all $n = 182$ nestlings in this experiment. Next, we used this approach in a separate model to measure the likelihood of nestlings to fledge for $n = 152$ nestlings that were alive at D12 (excluding those that were terminally collected). Finally, we used the same analytical approach to assess potentially longer-term effects of heat on the likelihood of nestlings to recruit to the population in following breeding seasons, focused on the $n = 145$ nestling that fledged.

Results

Temperature: Heat-exposed nests averaged 2.8°C hotter than controls (35.5°C ± 0.8, 38.3°C ± 0.7; Table 2). Our GAM explained 62% of the deviance in hourly nest temperature elevation, which was significantly higher in heated nests ($\beta = 0.16$, SE = 0.07, $t = 2.27$, $p = 0.02$), and in larger broods ($\beta = 0.10$, SE = 0.04, $t = 3.33$, $p = 0.001$). As expected for temperatures measured in the wild, temperature elevation varied non-linearly across experiment hour (effective degrees of freedom, EDF = 13.95, $F = 104.81$, $p < 0.0001$). The non-linear pattern across time also varied between treatments, with less temporal variation in heated nests (experiment hour*treatment: control: EDF = 12.28, $F = 3.85$, $p < 0.0001$; heat: EDF = 0.03, $F = 0.002$, $p < 0.0001$; Figure 1). This temporal variation seems to stem from diurnal variability (Figure 1), yet the heated nests remain hotter than controls throughout the experiment, with higher max and min temperatures (Table 2). Based on a post-hoc validation experiment (see S15a), our iButton orientation may have underestimated treatment-induced temperatures by as much as 2.7°C.

Behavior: Experimental heating significantly affected nestling thermoregulatory behaviors. We observed significantly more panting in heated nests ($\beta = 7.50$, SE = 2.22, $Z_{1,53} = 3.38$, $p = 0.001$) and in larger broods (brood size: $\beta = 0.87$, SE = 0.21, $Z_{1,53} = 4.21$, $p < 0.0001$); model $R^2m = 0.53$, $R^2c = 0.53$. Further this model showed significantly more panting at the later timepoint (timepoint: $\beta = 0.75$, SE = 0.21, $Z_{1,53} = 3.63$, $p < 0.0001$) and a significant treatment by timepoint interaction ($\beta = -0.73$, SE = 0.29, $Z_{1,53} = -2.52$, $p = 0.01$). This interaction stems from markedly more panting in heat-exposed vs. control nests on D6 (treatment: $\beta = 11.87$, SE = 5.22, $t_{1,56} = -2.27$, $p = 0.03$), but by D9, there is no difference between treatments (treatment: $\beta = 0.60$, SE = 5.42, $t_{1,56} = -0.11$, $p = 0.91$); Figure 2A. We observed significantly less huddling in heated nestlings (treatment: $\beta = -0.56$, SE = 0.23, $F_{1,30.3} = 6.12$, $p = 0.02$; Figure 2B) and in smaller broods (brood size: $\beta = 0.75$, SE = 0.10, $F_{1,29.23} = 60.03$, $p < 0.0001$); model $R^2m = 0.62$, $R^2c = 0.76$.

Experimental heating did not significantly affect other behaviors measured here. We observed significantly fewer nestlings begging in smaller broods ($\beta = 0.55$, SE = 0.09, $F_{1,32.58} = 37.32$, $p < 0.0001$) and when nestlings are younger ($\beta = 0.22$, SE = 0.05, $F_{1,29.59} = 23.13$, $p < 0.0001$), but there was no difference between treatments ($\beta = 0.07$, SE = 0.21, $F_{1,33.53} = 0.12$, $p = 0.73$, Figure S2); model $R^2m = 0.53$, $R^2c = 0.74$. Mean begging intensity was not affected by treatment ($\beta = -0.05$, SE = 0.10, $F_{1,59} = 0.2$, $p = 0.63$, Figure: S2); model $R^2m = 0.00$, $R^2c = 0.00$. For maternal visitation, model averaging showed no significant effect of treatment ($\beta = -21.65$, Adj. SE = 17.59, $Z = 1.23$, $p = 0.22$, Figure: S3A) and no significant effect of brood size ($\beta = -5.65$, Adj. SE = 8.26, $Z = 0.68$, $p = 0.49$); models $R^2m = 0.07$ -0.16 (Table S5-6). For paternal visitation, model averaging showed no significant of treatment ($\beta = 17.49$, Adj. SE = 22.96, $Z = 0.76$, $p = 0.45$, Figure: S3B) or ambient temperature ($\beta = 3.63$, Adj. SE = 8.67, $Z = 0.42$, $p = 0.68$); models $R^2m = 0.04$ -0.09 (Table S5-6).

Morphology: We observed significantly more growth in heated nests ($\beta = 59.18$, SE = 21.54, $F_{1,164.84} = 7.55$, $p = 0.01$), in warmer ambient temperatures ($\beta = 9.14$, SE = 2.60, $F_{1,34.55} = 12.61$, $p = 0.001$), in nestlings with smaller starting mass (D6 mass: $\beta = -11.74$, SE = 1.26, $F_{1,155.93} = 234.16$, $p < 0.0001$), and especially in heated nestlings with smaller starting mass (treatment*D6 mass: $\beta = -4.74$, SE = 1.84, $F_{1,156.01} = 6.61$, $p = 0.01$; Figure 3A). There was no effect of brood size ($\beta = 3.61$, SE = 3.30, $F_{1,34.33} = 1.2$, $p = 0.28$); model: $R^2m = 0.54$, $R^2c = 0.84$. Heated nestlings gained proportionally more mass than controls (D6-D12: heat mean = 96.1% ± 35.2, control mean = 90.1% ± 34.6), but this effect was strongest among smaller nestlings, as indicated by the significant treatment*D6 mass interaction (Figure 3A).

Wing length was significantly longer in heated nests (treatment: $\beta = 3.06$, SE = 1.50, $F_{1,33.32} = 4.36$, $p = 0.04$; Figure 3B), in larger broods ($\beta = 1.55$, SE = 0.65, $F_{1,36.7} = 5.65$, $p = 0.02$) and in warmer ambient temperature ($\beta = 1.5$, SE = 0.50, $F_{1,35.97} = 8.81$, $p = 0.005$); model: $R^2m = 0.2$, $R^2c = 0.58$. On average, heated nestling wings were 55.2 ± 3.8 mm at the end of the experiment, whereas controls were 51.8 ± 7.5 mm.

HSP gene expression: Experimental heating did not affect HSP gene expression in any tissue or timepoint sampled here (Figure 4; $R^2m = 0.00-0.09$). D6 blood HSP PC1 was not significantly affected by heat ($\beta = 0.13$, SE = 0.32, $F_{1,36.66} = 0.16$, $p = 0.69$). For D12 blood HSP PC1, model averaging indicated no significant effects of treatment ($\beta = -0.16$, Adj. SE = 0.35, $Z = 0.46$, $p = 0.65$) or brood size ($\beta = -0.14$, Adj. SE = 0.18, $Z = 0.79$, $p = 0.43$); see Table S5-6. For pectoral muscle HSP PC1, hippocampus HSP PC1, and ventromedial telencephalon HSP PC1, the focal model had the lowest ΔAIC . In each case, there was no significant effect of treatment (PM: $\beta = -0.1$, SE = 0.69, $F_{1,21} = 0.02$, $p = 0.89$; HPC: $\beta = -0.36$, SE = 0.60, $F_{1,22} = 0.37$, $p = 0.55$; VmT: $\beta = -0.27$, SE = 0.63, $F_{1,22} = 0.18$, $p = 0.68$). For hypothalamus HSP PC1, model averaging indicated no significant effects of treatment ($\beta = 0.04$, Adj. SE = 0.52, $Z = 0.09$, $p = 0.93$) or brood size ($\beta = 0.13$, Adj. SE = 0.20, $Z = 0.63$, $p = 0.53$); see Table S5-6.

Corticosterone: For both baseline Cort and handling-induced Cort, there was no significant effect of treatment (baseline: $\beta = -0.03$, SE = 0.10, $F_{1,35.19} = 0.09$, $p = 0.77$, $R^2m = 0.00$, $R^2c = 0.15$; handling-induced: $\beta = 0.11$, SE = 0.10, $F_{1,36} = 1.72$, $p = 0.20$, $R^2m = 0.04$, $R^2c = 0.56$); Figure 5.

SOD gene expression: We observed significantly lower D12 blood SOD mRNA expression in heated nests ($\beta = -4.33$, SE = 1.90, $F_{1,35} = 5.16$, $p = 0.03$; Figure 6A) and in warmer ambient temperatures ($\beta = -1.64$, $F_{1,35} = 9.0$, $p = 0.005$); model: $R^2m = 0.28$.

Telomere length in blood at D12: We observe no effect of heat on relative telomere length ($\beta = -0.03$, SE = 0.10, $F_{1,33.24} = 0.1$, $p = 0.75$; Figure 6B); model: $R^2m = 0.00$, $R^2c = 0.21$.

Likelihood of survival and recruitment: There was no treatment effect on the likelihood of nestlings to survive to D12 ($\beta = 0.98$, SE = 0.95, $Z = 1.03$, $p = 0.30$) or the likelihood to fledge ($\beta = -0.26$, SE = 1.01, $Z = -0.26$, $p = 0.80$). 174 of the 182 nestlings were alive on D12 ($n = 78$ heat and $n = 96$ control) and 145 out of 149 nestlings remaining after terminal collections successfully fledged ($n = 63$ heat and $n = 82$ control). In subsequent years, we recaptured 11 heated and 5 control nestlings that returned to breed in the population (4.9% of control and 13.8% of heat fledglings). This represented a marginal, but not significant, treatment effect on the likelihood of recruiting into the breeding population ($\beta = 1.24$, SE = 0.67, $Z = 1.85$, $p = 0.06$).

Discussion

We experimentally elevated nest temperatures by an average of 2.8°C, an effect that is comparable to expected temperature rises over the next 100 years (Hayhoe et al. 2009; Reidmiller et al. 2018). We documented increased panting, reduced huddling, and accelerated growth, the latter of which was most prominent among the initially smallest individuals. This accelerated development was not a byproduct of resource availability – heat did not affect nestling begging or parental visitation. Heat also did not affect heat shock protein gene expression in any of the five tissues sampled here. Other common physiological ‘stress’ responses were also not significantly different between treatments, including baseline corticosterone secretion, handling-induced corticosterone secretion, or relative telomere length. However, blood gene expression for the antioxidant SOD was lower in heated nestlings at the end of the experiment. Because SOD mitigates oxidative damage by neutralizing reactive oxygen species (Wang et al. 2018), this finding suggests that heated nestlings may be less equipped to cope with oxidative stress. None of these phenotypic effects translated to treatment differences in the immediate likelihood to survive; however, heated nestlings were marginally, but not significantly, more likely to recruit in following years. Thus, despite one apparent physiological cost, our multi-trait investigation demonstrates general resilience to a mild heatwave. Below, we discuss how behavioral buffering may shape persistence amidst rising temperatures, and more broadly, how organismal frameworks like ours may improve predictions of species resilience.

Thermoregulatory behaviors were robustly affected by our experimental heatwave. On the first day of the experiment (D6 post-hatch), we observed nearly 14-fold more panting in heat vs. control nests. However, this effect dissipated by the third day of the experiment (D9 post-hatch), when panting rates were comparable in heat and control nests. Comparing D6 to D9 among controls, we observed more panting as nestlings age, but nest temperatures did not increase during this time (see *Figure 1* and *Figure S4*). This natural increase in panting with age may relate to natural increases in body mass. Afterall, larger bodies retain more heat (Cramer & Jay 2016), and nestlings gained an average of 7.2g between D6 and D9 (68% increase in mass). Comparing heat and control nests on D6, we see more panting in heated nests. If heat continued to have a comparable or additive effect as the experimental heatwave continued, we would expect to again see more panting in hot nests vs. controls on D9. Instead, we see that heated nestlings converge to typical (control-like) amounts of panting by D9, despite sustained heat. Thus, our data are consistent with some degree of behavioral acclimation to heat. Acclimation generally occurs when individuals plastically adjust to their environment in adaptive ways (Rohr et al. 2018). Panting is a key heat dissipation behavior in passerines (Nord & Nilsson 2019) and, based on our findings, panting seems to be a first line of defense against inescapable heat.

Heated nestlings also huddled less than controls on both the first and third day of heat. Huddling is known to decrease heat loss (Gilbert et al. 2010), suggesting that reduced huddling may increase heat loss by reducing conductive heat transfer among siblings. To the degree that diminished huddling is a general response to heat (as seen in other wild and agricultural contexts: Huynh & Aarnink 2005; Shah et al. 2003), our warming climate may influence brood or litter sizes in the future, as there may be heat-related constraints on space use for animals confined to a fixed burrow or nest.

We found a positive effect of heat on nestling growth, despite no treatment effect on nestling begging or parental visitation. Further, the heat effect on mass gain was strongest in nestlings that were initially the smallest. We speculate that our heat treatment may have protected nestlings from cold temperatures overnight, particularly since hourly nest temperatures in heated nests never dipped below 24.2°C overnight (*Table 2*). This difference may be biologically important given that 22°C can induce cold stress (Węgrzyn 2013), and cold weather affects nestling growth (Shipley et al. 2020). Though control nests did reach temperatures above the projected thermoneutral zone, this occurred less frequently than in heated nests (*Table 2*). Thus, at least in temperate climates where spring and summer nights can be cool, heatwaves may allow smaller animals to better allocate energy towards growth or other critical processes. Organismal approaches that quantify multiple traits at various times during a heatwave are essential to interpreting the valence of these effects.

We expected heat to induce physiological responses that counteract negative effects of heat. Among these mechanisms, HSPs are of interest because they represent a conserved response to stress-induced cellular damage (Sørensen et al. 2003). Though there are few studies on HSPs in songbirds, there is evidence that nestlings are able to upregulate HSPs in response to stressors (Sørensen et al. 2003), though these effects may differ among tissues (Lipshutz et al. 2022). Our thorough analysis of three HSPs across five tissues and two time-points during heat exposure provides a compelling null result. These results strongly suggest that mild heat did not elicit HSP responses, at least when coupled with apparent behavioral acclimation. Behavior has long been considered a first line of defense to novel environmental challenges (West-Eberhard 2003). We extend this idea here in animals with limited mobility in a fixed ecological niche. The central role of behavior in dealing with heat is underscored by the null results we found for a battery of physiological stress responses.

For instance, glucocorticoid hormones are commonly engaged during metabolic challenges, yet we found no treatment effect on baseline or handling-induced Cort concentrations. Baseline Cort is important for maintaining homeostasis and can be elevated under prolonged metabolic challenges,

whereas acute Cort elevation, in this case induced by a standardized restraint, is an adaptive response to mobilize resources (Mentesana et al. 2022; Romero et al. 2009). The lack of glucocorticoid differences here may suggest that our experimental heatwave was not hot enough to be metabolically challenging.

Alternatively, we note that experimentally generated nest temperatures are above the thermoneutral zone of similarly sized passerines (Khaliq et al. 2014), and our iButton orientation may have underestimated our heat effect (see *S1S1a*). There is debate on exactly how to measure thermoneutral zones in broadly applicable ways, considering that results may vary between captive and field settings, across developmental time, and among breeding stages (reviewed in Mitchell et al. 2018). Indeed, even for the well-characterized zebra finch (*Taeniopygia guttata*), the upper limit of thermoneutrality ranges from 35.9 to 40°C (Calder 1964; Marschall & Prinzinger 1991; Wojciechowski et al. 2021). The thermoneutral zone for young tree swallows has not yet been determined, but, using the average for similarly sized passerines, we note that heated nests experienced more time in temperatures above the upper limit (*Table 2*). Control nests also experience temperatures above the projected thermoneutral zone, but max temperatures in focal nests were markedly higher in the heat-exposed nests (*Table 2*), underscoring the importance of examining the effects of inescapable heat in microclimates that exceed ambient temperatures (Cunningham et al. 2021).

Therefore, our results suggest that perhaps tree swallows are relatively robust to mild heatwaves. Many previous studies on heat effects in nestlings, have shown null or negative effects (Andreasson et al. 2018; Andrew et al. 2017; Corregidor-Castro et al. 2021; Hsu et al. 2020; Rodriguez et al. 2016; Ton et al. 2021), but notably, Dawson et al. (2005) used a similar air-activated warmer with tree swallows and found that heated nestlings were heavier and had faster feather growth after 12 days of heat exposure, albeit in a cooler northern environment. Tree swallows have also recently expanded their breeding range southward into the hot and humid eastern United States (McCaslin & Heath 2020), contrary to northward range shifts seen in hundreds of other species (Root et al. 2003). To the degree that tree swallows are ‘winners’ amidst rising temperatures, their apparent ability to behaviorally acclimate could contribute to resilience to rising temperatures. Understanding interspecific differences in resilience to effects of climate change is an active area of research (e.g., Cohen et al. 2020; Huang et al. 2023; Nguyen et al. 2022; Ujszegi et al. 2022), and our results extend these ideas in a species whose populations are growing in warmer climates (Shutler et al. 2012; Siefferman et al. 2023; Wright et al. 2019).

Supporting this view, we found that heat had null or marginally positive effects on metrics of longevity and recruitment. Previous studies have shown that early-life stressors can affect telomere length (Eastwood et al. 2022; Wolf et al. 2021), and prolonged heat exposure can shorten telomeres and reduce longevity (Zhang et al. 2018). Yet here, six days of heat did not alter relative telomere length. Heated nestlings were also marginally more likely to recruit into the population in subsequent years. Though the sample size of recruited birds is small, this trend suggests a positive effect of mild heat.

Even among apparently heat-resilient species, there may still be sub-lethal effects of heat. For instance, our heatwave did affect SOD gene expression, which was significantly lower in the blood of heat-exposed nestlings compared to controls at the end of the experiment. SOD encodes an enzyme that is central to inactivating free radicals that would otherwise generate oxidative damage (Wang et al. 2018). The dampening of SOD mRNA abundance is consistent with observations of lower enzyme activity, including that of SOD, after periods of heat stress (Zhao et al. 2022). If transient, these effects may interfere with the ability to cope with co-occurring stressors; if these effects endure beyond a heatwave, they may have more significant repercussions, particularly as heatwaves increase in frequency in our warming world.

Conclusion

As climate change intensifies, more species will encounter previously unseen exposure to heat. Organizations are looking for solutions to this problem, with some advocating that we prioritize the likely ‘winners’ of global change (e.g., Cornwall 2018; Gilbert et al. 2020), though debate remains on how best to manage these controversial decisions (e.g., Chapron et al. 2018; Wiedenfeld et al. 2021). Models and big data have been instrumental in evaluating some factors that characterize ‘winners’ – including ecological niches, population dynamics, and life history traits (e.g., Fischer & Huth 2019; Marolla et al. 2021). We believe predictions that also integrate behavioral and physiological response mechanisms are critical for improving these analyses and associated conservation decisions. Our organismal approach, which quantifies a number of heat-response mechanisms across the animal and how they vary over time, provides such a framework, which we hope will improve understanding of species persistence in the face of climate change.

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Ethics

All methods were approved by the Indiana University, Bloomington Institutional Animal Care and Use Committee (IU protocol #18-004-16) and conducted with appropriate state and federal permits.

Tables

Table 1: Sample size per measure for each treatment group. Behavior was quantified at the nest level; therefore, the nestling sample size is indicated as NA.

Measures	Treatment (N)			
	Control		Heat	
	Nests	Nestlings	Nests	Nestlings
Nestling Behavior (D6)	16	NA	15	NA
Nestling Behavior (D9)	16	NA	13	NA
Maternal Visitation	10	NA	13	NA
Paternal Visitation	10	NA	12	NA
D6 BL HSP	21	40	17	33
D12 BL HSP	21	38	17	33
PM HSP	10	10	13	13
HPC HSP	11	11	13	13
HYPO HSP	12	12	11	11
VmT HSP	11	11	13	13
Baseline Cort	21	36	17	28
Handling-induced Cort	20	38	17	28
Telomere Length	21	55	17	47
D12 BL SOD	21	21	17	17
% Change in Mass	21	94	17	77
D12 WL	21	94	17	76
Survive to D12	21	96	17	78
Survive to Fledge	21	82	17	63
Recruitment	4	5	8	11

560 **Table 2:** Treatment effects on hourly nest temperatures. Means ($\pm$ standard error) come from hourly
 561 values, averaged per box. Percent of hours with nest temperature above 36.9°C is a proxy for the percent
 562 of hours above the average upper limit of the thermoneutral zone in similarly sized passerines (36.9°C,
 563 extracted from Table S1 in Khaliq et al. 2014).

Temperature Summary	Control	Heat
Mean nest temp	35.5°C $\pm$ 0.8	38.3°C $\pm$ 0.7
Min nest temp	26.8°C $\pm$ 1.2	30.0°C $\pm$ 1.2
Max nest temp	40.2°C $\pm$ 0.3	42.9°C $\pm$ 0.8
Mean ambient temp	21.3°C $\pm$ 0.3	21.8°C $\pm$ 0.4
Mean nest temp elevation above ambient	14.3°C $\pm$ 0.7	16.5°C $\pm$ 0.8
% hours with nest temp > 36.9°C	51.6% $\pm$ 7.1	74.5% $\pm$ 7.4
Daytime nest temp range (7:01 – 20:59)	18 – 41.5°C	21.5 – 50.7°C
Overnight nest temp range (21:00 - 7:00)	15.5 - 41.8°C	24.2 – 48.0°C

564

Figures

Figure 1: Nest temperature elevation above ambient (°C) across the duration of the experiment; ambient temperature averaged $21.5^{\circ}\text{C} \pm 0.1$. Each point represents one hour per nest. Shading indicates 95% CI per treatment. Generalized additive model uses a smoothed function and controls for the random effect of nest.

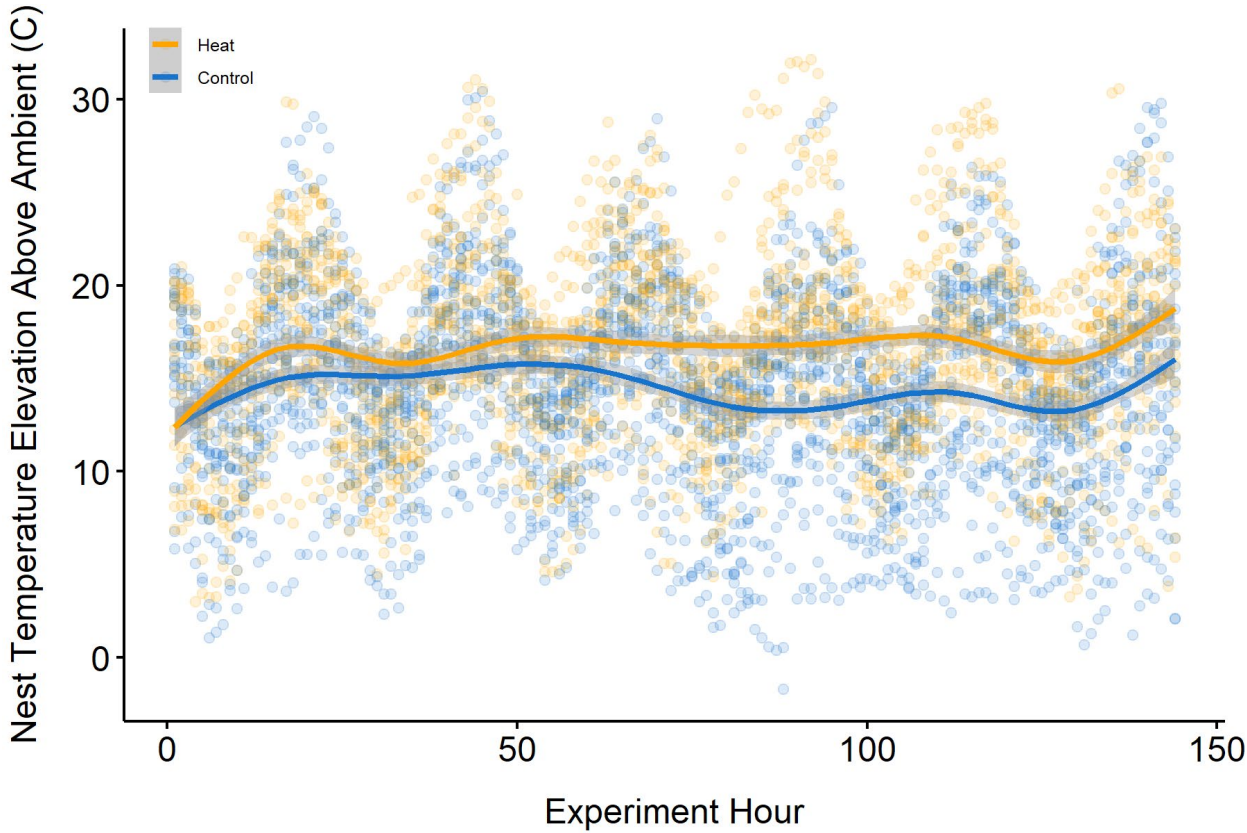


Figure 2: (A) Total duration (minutes) any nestling was observed panting and (B) mean number of nestlings huddling during the 1h observation period. Each point represents one nest. Lines connect nests observed on D6 and D9 timepoints. Unconnected points represent nests for which we had only one day of complete video data. Mean line per treatment is shown in black.

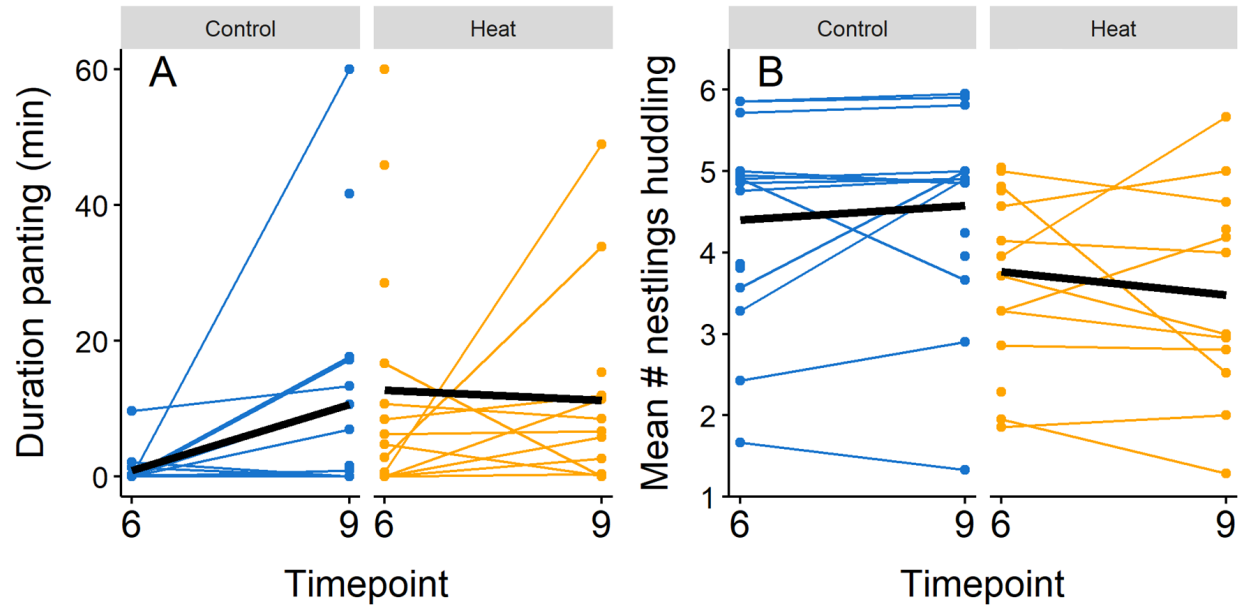


Figure 3: (A) Nestling growth (percent change in nestling mass across the experiment), relative to starting mass at D6. (B) D12 nestling wing length (mm) at the end of the experiment. Error bars are mean $\pm$ SE. Shading indicates 95% CI. Each point represents one nestling. Models control for the random effect of nest.

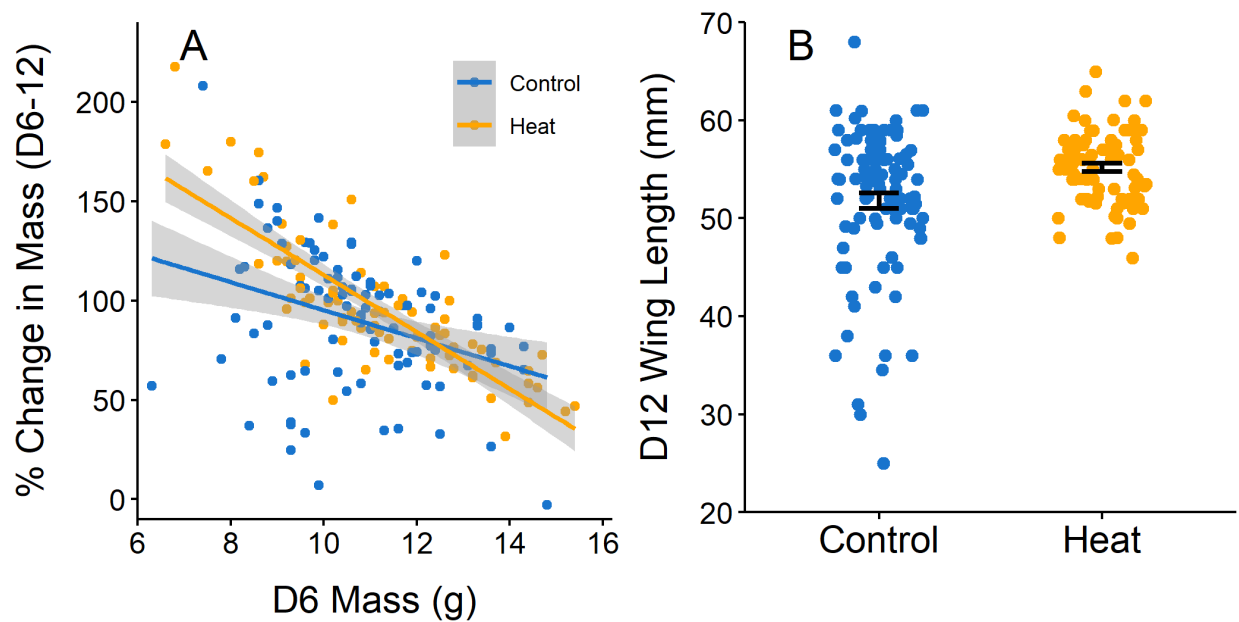


Figure 4: Heat shock protein gene expression was unaffected by treatment. Y-axes represent the first principal component derived from relative gene expression of three heat shock proteins, per tissue. (A) D6 Blood HSP PC1; (B) D12 Blood HSP PC1; (C) Pectoral Muscle (PM) HSP PC1; (D) Hippocampus (HPC) HSP PC1; (E) Hypothalamus (HYPO) HSP PC1; (F) Ventromedial Telencephalon (VmT) HSP PC1. Each point represents one nestling. Blood models account for the random effect of nest. Error bars are mean $\pm$ SE.

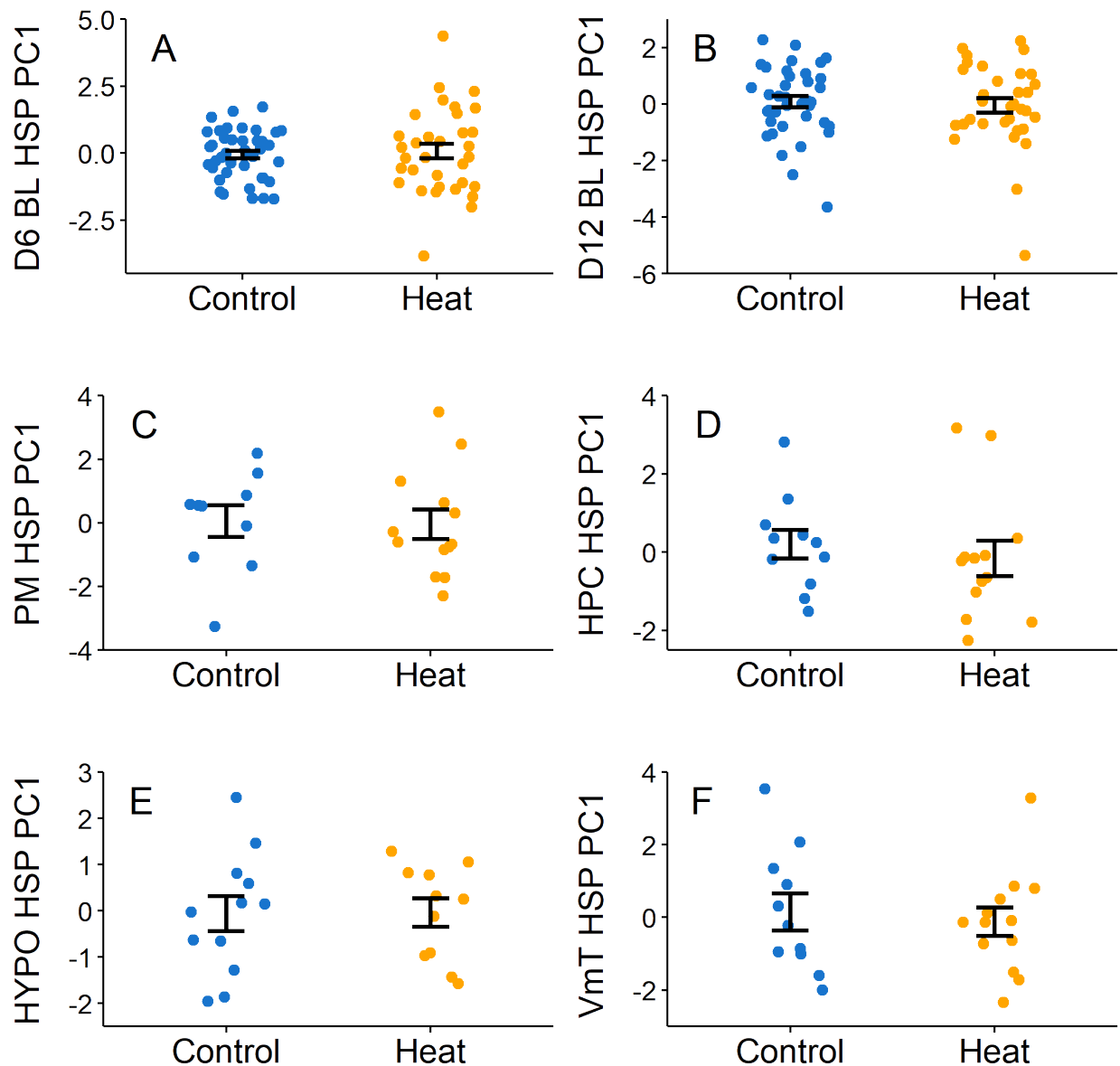
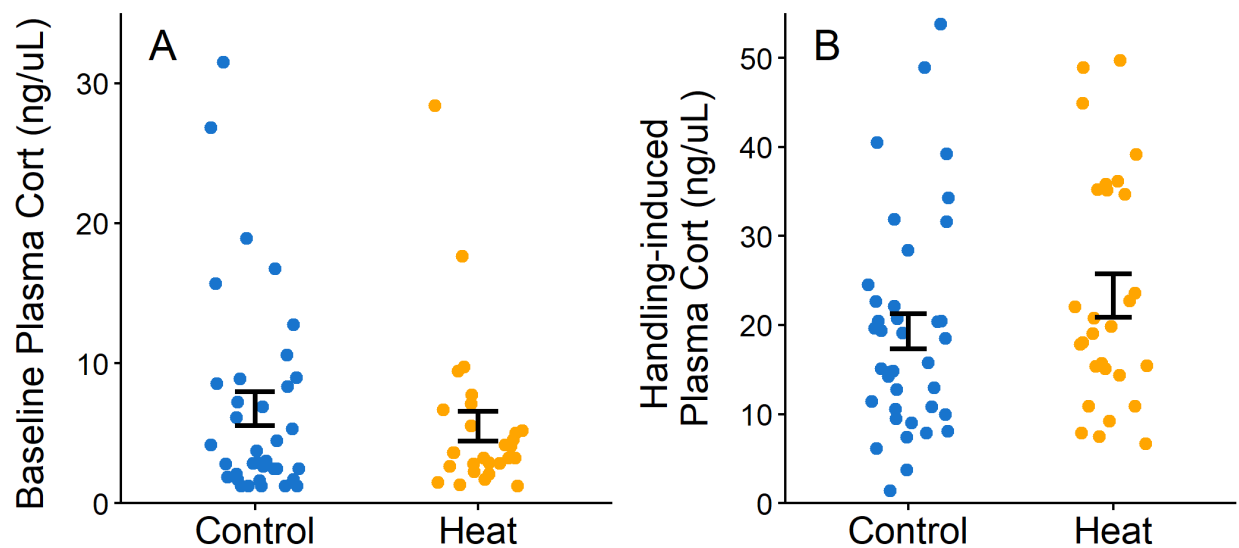
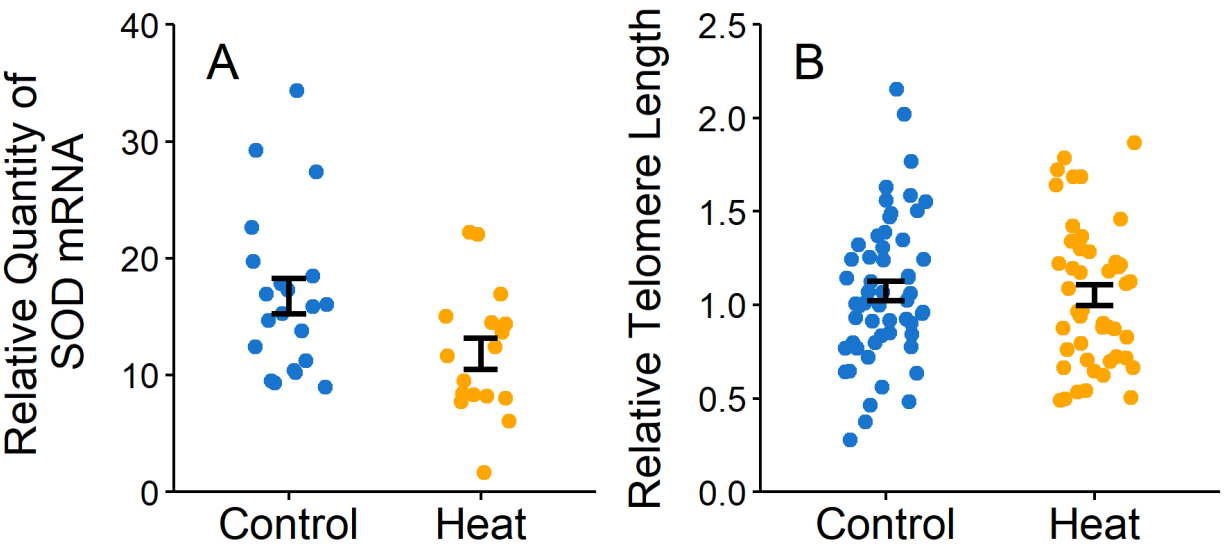


Figure 5: (A) Baseline and (B) handling-induced plasma corticosterone (Cort) concentration (ng/μL) were not significantly affected at the end of the experimental heatwave. Each point represents one nestling, and models account for the random effect of nest. Error bars are mean $\pm$ SE.



596 **Figure 6:** (A) SOD relative gene expression ($2^{-\Delta Ct}$) and (B) relative telomere length ($2^{-\Delta\Delta Ct}$) in D12 blood
597 across treatments. Telomere length models account for the random effect of nest. Each point represents
598 one nestling. Error bars are mean $\pm$ SE.



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References

- Andreasson, F., Nord, A., & Nilsson, J.-Å. (2018). Experimentally increased nest temperature affects body temperature, growth and apparent survival in blue tit nestlings. *Journal of Avian Biology*, 49(2). doi:10.1111/jav.01620
- Andrew, S., Hurley, L., Mariette, M., & Griffith, S. (2017). Higher temperatures during development reduce body size in the zebra finch in the laboratory and in the wild. *Journal of evolutionary biology*, 30(12), 2156.
- Angilletta, M. J. (2009). Thermal adaptation: A theoretical and empirical synthesis. *Oxford University Press*. doi:<https://doi.org/10.1093/acprof:oso/9780198570875.001.1>
- Barton, K. (2019). Mumin: Multi-model inference. Retrieved from <https://cran.R-project.org/package=mumi>.
- Bentz, A., Thomas, G. W., Rusch, D. B., & Rosvall, K. A. (2019). Tissue-specific expression profiles and positive selection analysis in the tree swallow (*tachycineta bicolor*) using a de novo transcriptome assembly. *Scientific Reports*, 9(1), 1.
- Bingman, V. P., Hough li, G. E., Kahn, M. C., & Siegel, J. J. (2003). The homing pigeon hippocampus and space: In search of adaptive specialization. *Brain, Behavior and Evolution*, 62(2), 117. doi:10.1159/000072442
- Blanckenhorn, W. U. (2000). The evolution of body size: What keeps organisms small? *The Quarterly Review of Biology*, 75(4), 385. doi:10.1086/393620
- Blumstein, D. T., & Daniel, J. C. (2007). *Quantifying behavior the jwatcher way*: Sinauer Associates, Incorporated.
- Bourne, A. R., Ridley, A. R., McKechnie, A. E., Spottiswoode, C. N., & Cunningham, S. J. (2021). Dehydration risk is associated with reduced nest attendance and hatching success in a cooperatively breeding bird, the southern pied babbler *turdoides bicolor*. *Conservation physiology*, 9(1), coab043. doi:10.1093/conphys/coab043
- Burnham, K., & Anderson, D. R. (2002). Model selection and multimodel inference. *New York: Springer*.
- Calder, W. A. (1964). Gaseous metabolism and water relations of the zebra finch, *taeniopygia castanotis*. *Physiological Zoology*, 37(4), 400.
- Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative pcr method. *Nucleic acids research*, 37(3), e21.
- Chapron, G., Epstein, Y., & López-Bao, J. V. (2018). Us bill illustrates how conservation triage can lead to extinctions. *Nature*, 554(7692).
- Chatelain, M., Drobniak, S. M., & Szulkin, M. (2020). The association between stressors and telomeres in non-human vertebrates: A meta-analysis. *Ecology letters*, 23(2), 381.
- Cheng, C.-H., Guo, Z.-X., Luo, S.-W., & Wang, A.-L. (2018). Effects of high temperature on biochemical parameters, oxidative stress, DNA damage and apoptosis of pufferfish (*takifugu obscurus*). *Ecotoxicology and Environmental Safety*, 150, 190. doi:<https://doi.org/10.1016/j.ecoenv.2017.12.045>
- Choy, E. S., O'Connor, R. S., Gilchrist, H. G., Hargreaves, A. L., Love, O. P., Vézina, F., & Elliott, K. H. (2021). Limited heat tolerance in a cold-adapted seabird: Implications of a warming arctic. *Journal of Experimental Biology*, 224(13). doi:10.1242/jeb.242168
- Cohen, J. M., Fink, D., & Zuckerman, B. (2020). Avian responses to extreme weather across functional traits and temporal scales. *Global Change Biology*, 26(8), 4240. doi:<https://doi.org/10.1111/gcb.15133>
- Cornwall, W. (2018). Should it be saved? *Science*, 361(6406), 962. doi:doi:10.1126/science.361.6406.962
- Corregidor-Castro, A., & Jones, O. R. (2021). The effect of nest temperature on growth and survival in juvenile great tits *parus major*. *Ecology and Evolution*.

- Cramer, M. N., & Jay, O. (2016). Biophysical aspects of human thermoregulation during heat stress. *Autonomic Neuroscience*, 196, 3.
- Cunningham, S. J., Gardner, J. L., & Martin, R. O. (2021). Opportunity costs and the response of birds and mammals to climate warming. *Frontiers in Ecology and the Environment*, 19(5), 300. doi:<https://doi.org/10.1002/fee.2324>
- Dawson, R. D., Lawrie, C. C., & O'Brien, E. L. (2005). The importance of microclimate variation in determining size, growth and survival of avian offspring: Experimental evidence from a cavity nesting passerine. *Oecologia*, 144(3), 499.
- du Plessis, K. L., Martin, R. O., Hockey, P. A. R., Cunningham, S. J., & Ridley, A. R. (2012). The costs of keeping cool in a warming world: Implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Global Change Biology*, 18(10), 3063. doi:<https://doi.org/10.1111/j.1365-2486.2012.02778.x>
- Eastwood, J. R., Connallon, T., Delhey, K., Hall, M. L., Teunissen, N., Kingma, S. A., . . . Peters, A. (2022). Hot and dry conditions predict shorter nestling telomeres in an endangered songbird: Implications for population persistence. *Proceedings of the National Academy of Sciences*, 119(25), e2122944119.
- Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology*, 61, 243. doi:DOI 10.1146/annurev.physiol.61.1.243
- Fischer, E. M., Sippel, S., & Knutti, R. (2021). Increasing probability of record-shattering climate extremes. *Nature Climate Change*, 11(8), 689. doi:10.1038/s41558-021-01092-9
- Fischer, S. M., & Huth, A. (2019). An approach to study species persistence in unconstrained random networks. *Scientific Reports*, 9(1), 14110. doi:10.1038/s41598-019-50373-z
- Fontella, F. U., Siqueira, I. R., Vasconcellos, A. P. S., Tabajara, A. S., Netto, C. A., & Dalmaz, C. (2005). Repeated restraint stress induces oxidative damage in rat hippocampus. *Neurochemical Research*, 30(1), 105. doi:10.1007/s11064-004-9691-6
- Fox, J., & Weisberg, S. (2018). *An r companion to applied regression*: Sage publications.
- Gaunt, A. S., Oring, L. W., Able, K., Anderson, D., Baptista, L., Barlow, J., & Wingfield, J. (2010). Guidelines to the use of wild birds in research. In (3rd ed.): Washington, DC: The Ornithological Council.
- Gebhardt-Henrich, S., & Richner, H. (1998). Causes of growth variation and its consequences for fitness. In *Avian growth and development — evolution within the altricial-precocial spectrum* (pp. 324). New York: Oxford University Press.
- Gilbert, C., McCafferty, D., Le Maho, Y., Martrette, J.-M., Giroud, S., Blanc, S., & Ancel, A. (2010). One for all and all for one: The energetic benefits of huddling in endotherms. *Biological Reviews*, 85(3), 545. doi:<https://doi.org/10.1111/j.1469-185X.2009.00115.x>
- Gilbert, S. L., Broadley, K., Doran-Myers, D., Droghini, A., Haines, J. A., Härmäläinen, A., . . . Boutin, S. (2020). Conservation triage at the trailing edge of climate envelopes. *Conservation Biology*, 34(1), 289. doi:<https://doi.org/10.1111/cobi.13401>
- Goodson, J. L. (2005). The vertebrate social behavior network: Evolutionary themes and variations. *Hormones and behavior*, 48(1), 11.
- Hayhoe, K., VanDorn, J., Naik, V., & Wuebbles, D. (2009). Climate change in the midwest: Projections of future temperature and precipitation. Cambridge, MA: Union of Concerned Scientists Online at http://www.ucsusa.org/assets/documents/global_warming/midwest-climate-impacts.pdf, accessed January, 26, 2014.
- Heo, S., Bell, M. L., & Lee, J.-T. (2019). Comparison of health risks by heat wave definition: Applicability of wet-bulb globe temperature for heat wave criteria. *Environmental Research*, 168, 158. doi:<https://doi.org/10.1016/j.envres.2018.09.032>

- Hoffmann, A. A., Sørensen, J. G., & Loeschcke, V. (2003). Adaptation of drosophila to temperature extremes: Bringing together quantitative and molecular approaches. *Journal of thermal Biology*, 28(3), 175.
- Hsu, B.-Y., Sarraude, T., Cossin-Sevrin, N., Crombecque, M., Stier, A., & Ruuskanen, S. (2020). Testing for context-dependent effects of prenatal thyroid hormones on offspring survival and physiology: An experimental temperature manipulation. *Scientific Reports*, 10(1), 1.
- Huang, Q., Bateman, B. L., Michel, N. L., Pidgeon, A. M., Radeloff, V. C., Heglund, P., . . . Sauer, J. R. (2023). Modeled distribution shifts of north american birds over four decades based on suitable climate alone do not predict observed shifts. *Science of The Total Environment*, 857, 159603. doi:<https://doi.org/10.1016/j.scitotenv.2022.159603>
- Huey, R. B., Hertz, Paul E., & Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: A null model approach. *The American Naturalist*, 161(3), 357. doi:10.1086/346135
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), 1665.
- Huynh, T., & Aarnink, A. (2005). Heat stress in pigs. *Pig Progress*, 21(3), 30.
- Jiang, J., Wu, S., Liu, X., Wang, Y., An, X., Cai, L., & Zhao, X. (2015). Effect of acetochlor on transcription of genes associated with oxidative stress, apoptosis, immunotoxicity and endocrine disruption in the early life stage of zebrafish. *Environmental Toxicology and Pharmacology*, 40(2), 516. doi:<https://doi.org/10.1016/j.etap.2015.08.005>
- Kang, D., & Shim, K. (2021). Early heat exposure effect on the heat shock proteins in broilers under acute heat stress. *Poultry Science*, 100(3), 100964. doi:<https://doi.org/10.1016/j.psj.2020.12.061>
- Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., & Pfenninger, M. (2014). Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 281(1789), 20141097. doi:doi:10.1098/rspb.2014.1097
- Kingsolver, J. G., Moore, M. E., Hill, C. A., & Augustine, K. E. (2020). Growth, stress, and acclimation responses to fluctuating temperatures in field and domesticated populations of manduca sexta. *Ecology and Evolution*, 10(24), 13980.
- Larson, E. R., Eastwood, J. R., Buchanan, K. L., Bennett, A. T., & Berg, M. L. (2015). How does nest box temperature affect nestling growth rate and breeding success in a parrot? *Emu*, 115(3), 247.
- Lendvai, A. Z., Akçay, Ç., Ouyang, J. Q., Dakin, R., Domalik, A. D., St John, P. S., . . . Bonier, F. (2015). Analysis of the optimal duration of behavioral observations based on an automated continuous monitoring system in tree swallows (*tachycineta bicolor*): Is one hour good enough? *PLoS ONE*, 10(11), e0141194.
- Lindquist, S., & Craig, E. A. (1988). The heat-shock proteins. *Annual review of genetics*, 22(1), 631.
- Lipshutz, S. E., Howell, C. R., Buechlein, A. M., Rusch, D. B., Rosvall, K. A., & Derryberry, E. P. (2022). How thermal challenges change gene regulation in the songbird brain and gonad: Implications for sexual selection in our changing world. *Molecular Ecology*, 31, 3613.
- Lombardo, M. P., Thorpe, P. A., Otieno, S., Hawker, A., Welgarz, D., Andrews, D., & Black, A. (2020). Yearly variation in factors associated with local recruitment of tree swallows. *Journal of Field Ornithology*, 91(2), 199.
- Loughran, C. L., & Wolf, B. O. (2020). The functional significance of panting as a mechanism of thermoregulation and its relationship to the critical thermal maxima in lizards. *Journal of Experimental Biology*, 223(17). doi:10.1242/jeb.224139
- Marolla, F., Henden, J.-A., Fuglei, E., Pedersen, Å. Ø., Itkin, M., & Ims, R. A. (2021). Iterative model predictions for wildlife populations impacted by rapid climate change. *Global Change Biology*, 27(8), 1547. doi:<https://doi.org/10.1111/gcb.15518>

- Marschall, U., & Prinzinger, R. (1991). Comparative ecophysiology of five finch species (estrildidae). *Journal of Ornithology*, 132(3), 319. doi:10.1007/BF01640540
- Marsh, R. L. (1980). Development of temperature regulation in nestling tree swallows. *The Condor*, 82(4), 461.
- McCarty, J. P. (2001). Variation in growth of nestling tree swallows across multiple temporal and spatial scales. *The Auk*, 118(1), 176.
- McCaslin, H. M., & Heath, J. A. (2020). Patterns and mechanisms of heterogeneous breeding distribution shifts of north american migratory birds. *Journal of Avian Biology*, 51(3).
- McKechnie, A. E., & Wolf, B. O. (2019). The physiology of heat tolerance in small endotherms. *Physiology*, 34(5), 302.
- Mentesana, L., & Hau, M. (2022). Glucocorticoids in a warming world: Do they help birds to cope with high environmental temperatures? *Hormones and Behavior*, 142, 105178. doi:<https://doi.org/10.1016/j.yhbeh.2022.105178>
- Mikami, S.-i. (1986). Immunocytochemistry of the avian hypothalamus and adenohypophysis. *International Review of Cytology*, 103, 189.
- Mitchell, D., Snelling, E. P., Hetem, R. S., Maloney, S. K., Strauss, W. M., & Fuller, A. (2018). Revisiting concepts of thermal physiology: Predicting responses of mammals to climate change. *Journal of Animal Ecology*, 87(4), 956. doi:<https://doi.org/10.1111/1365-2656.12818>
- Muñoz, M. M. (2022). The bogert effect, a factor in evolution. *Evolution*, 76(s1), 49. doi:10.1111/evo.14388
- Murugesan, S., Ullengala, R., & Amirthalingam, V. (2017). Heat shock protein and thermal stress in chicken. In *Heat shock proteins in veterinary medicine and sciences* (pp. 179): Springer.
- Nguyen, D., Masasa, M., Ovadia, O., & Guttman, L. (2022). Ecological insights into the resilience of marine plastisphere throughout a storm disturbance. *Science of The Total Environment*, 159775. doi:<https://doi.org/10.1016/j.scitotenv.2022.159775>
- Nord, A., & Nilsson, J. Å. (2019). Heat dissipation rate constrains reproductive investment in a wild bird. *Functional Ecology*, 33(2), 250.
- O'Connell, L. A., & Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science*, 336(6085), 1154.
- Oswald, S. A., Bearhop, S., Furness, R. W., Huntley, B., & Hamer, K. C. (2008). Heat stress in a high-latitude seabird: Effects of temperature and food supply on bathing and nest attendance of great skuas catharacta skua. *Journal of Avian Biology*, 39(2), 163. doi:<https://doi.org/10.1111/j.2008.0908-8857.04187.x>
- Pandolfi, J. M., Connolly, S. R., Marshall, D. J., & Cohen, A. L. (2011). Projecting coral reef futures under global warming and ocean acidification. *Science*, 333(6041), 418.
- Perez, J. H., Ardia, D. R., Chad, E. K., & Clotfelter, E. D. (2008). Experimental heating reveals nest temperature affects nestling condition in tree swallows (tachycineta bicolor). *Biology Letters*, 4(5), 468. doi:10.1098/rsbl.2008.0266
- Pilz, K. M., Quiroga, M. n., Schwabl, H., & Adkins-Regan, E. (2004). European starling chicks benefit from high yolk testosterone levels during a drought year. *Hormones and Behavior*, 46(2), 179.
- Pravosudov, V. V., Kitaysky, A. S., & Omanska, A. (2006). The relationship between migratory behaviour, memory and the hippocampus: An intraspecific comparison. *Proceedings of the Royal Society B: Biological Sciences*, 273(1601), 2641.
- Reidmiller, D. R., Avery, C. W., Easterling, D. R., Kunkel, K. E., Lewis, K. L. M., Maycock, T. K., & Stewart, B. C. (2018). Usgcrp: Impacts, risks, and adaptation in the united states: Fourth national climate assessment, volume ii. *US Global Change Research Program, Washington, DC, USA*. doi:doi:10.7930/NCA4.2018

- Rodriguez, S., & Barba, E. (2016). Nestling growth is impaired by heat stress: An experimental study in a mediterranean great tit population. *Zoological Studies*, 55, 40.
- Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology letters*, 21(9), 1425.
- Romero, L. M., Dickens, M. J., & Cyr, N. E. (2009). The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Hormones and behavior*, 55(3), 375.
- Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 140(1), 73.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., & Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, 421(6918), 57. doi:10.1038/nature01333
- Sales, K., Vasudeva, R., & Gage, M. J. G. (2021). Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *Royal Society Open Science*, 8(3), 201717. doi:doi:10.1098/rsos.201717
- Sánchez-Hidalgo, A. C., Muñoz, M. F., Herrera, A. J., Espinosa-Oliva, A. M., Stowell, R., Ayala, A., . . . de Pablos, R. M. (2016). Chronic stress alters the expression levels of longevity-related genes in the rat hippocampus. *Neurochemistry International*, 97, 181.
doi:<https://doi.org/10.1016/j.neuint.2016.04.009>
- Shah, B., Shine, R., Hudson, S., & Kearney, M. (2003). Sociality in lizards: Why do thick-tailed geckos (*nephurus milii*) aggregate? *Behaviour*, 140(8), 1039.
- Shipley, J. R., Twining, C. W., Taff, C. C., Vitousek, M. N., Flack, A., & Winkler, D. W. (2020). Birds advancing lay dates with warming springs face greater risk of chick mortality. *Proceedings of the National Academy of Sciences*, 202009864. doi:10.1073/pnas.2009864117
- Shutler, D., Clark, R. G., Fehr, C., & Diamond, A. W. (2006). Time and recruitment costs as currencies in manipulation studies on the costs of reproduction. *Ecology*, 87(11), 2938.
- Shutler, D., Hussell, D. J. T., Norris, D. R., Winkler, D. W., Robertson, R. J., Bonier, F., . . . Stanback, M. T. (2012). Spatiotemporal patterns in nest box occupancy by tree swallows across north america. *Avian Conservation and Ecology*, 7(1). doi:10.5751/ace-00517-070103
- Siefferman, L., Bentz, A. B., & Rosvall, K. A. (2023). Decoupling pioneering traits from latitudinal patterns in a north american bird experiencing a southward range shift. *Journal of Animal Ecology*, n/a(n/a), 0. doi:<https://doi.org/10.1111/1365-2656.13907>
- Silva, P. S., Hooper, H. B., Manica, E., Merighe, G. K. F., Oliveira, S. A., Traldi, A. S., & Negrão, J. A. (2021). Heat stress affects the expression of key genes in the placenta, placental characteristics, and efficiency of saanen goats and the survival and growth of their kids. *Journal of Dairy Science*, 104(4), 4970. doi:<https://doi.org/10.3168/jds.2020-18301>
- Sørensen, J. G., Kristensen, T. N., & Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, 6(11), 1025. doi:10.1046/j.1461-0248.2003.00528.x
- Stearns, S. C. (1992). *The evolution of life histories* (Vol. 249): Oxford university press Oxford.
- Ton, R., Stier, A., Cooper, C. E., & Griffith, S. C. (2021). Effects of heat waves during post-natal development on mitochondrial and whole body physiology: An experimental study in zebra finches. *Frontiers in physiology*, 12, 554.
- Tu, W.-L., Cheng, C.-Y., Wang, S.-H., Tang, P.-C., Chen, C.-F., Chen, H.-H., . . . Huang, S.-Y. (2016). Profiling of differential gene expression in the hypothalamus of broiler-type taiwan country chickens in response to acute heat stress. *Theriogenology*, 85(3), 483.
- Ujszegi, J., Bertalan, R., Ujhegyi, N., Verebelyi, V., Nemesházi, E., Mikó, Z., . . . Hettyey, A. (2022). “Heat waves” experienced during larval life have species-specific consequences on life-history traits and sexual development in anuran amphibians. *Science of The Total Environment*, 835, 155297. doi:<https://doi.org/10.1016/j.scitotenv.2022.155297>

- van Zanten, M., Ai, H., & Quint, M. (2021). Plant thermotropism: An underexplored thermal engagement and avoidance strategy. *Journal of Experimental Botany*.
- Verzuh, T. L., Hall, L. E., Cufau, T., Knox, L., Class, C., & Monteith, K. L. (2021). Behavioural flexibility in a heat-sensitive endotherm: The role of bed sites as thermal refuges. *Animal Behaviour*, 178, 77. doi:<https://doi.org/10.1016/j.anbehav.2021.05.020>
- Virgin, E. E., & Rosvall, K. A. (2018). Endocrine-immune signaling as a predictor of survival: A prospective study in developing songbird chicks. *General and Comparative Endocrinology*, 267, 193. doi:<https://doi.org/10.1016/j.ygcen.2018.08.008>
- Vitousek, M. N., Johnson, M. A., Downs, C. J., Miller, E. T., Martin, L. B., Francis, C. D., . . . Hau, M. (2019). Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels. *The American Naturalist*, 193(6), 866.
- Wan, Y., Ma, C., Wei, P., Fang, Q., Guo, X., Zhou, B., & Jiang, R. (2017). Dynamic expression of hsp90b1 mrna in the hypothalamus of two chinese chicken breeds under heat stress and association analysis with a snp in huainan chickens. *Czech Journal of Animal Science*, 62(2), 82.
- Wang, S. H., Cheng, C.-Y., Tang, P.-C., Chen, C.-F., Chen, H.-H., Lee, Y.-P., & Huang, S.-Y. (2015). Acute heat stress induces differential gene expressions in the testes of a broiler-type strain of taiwan country chickens. *PLoS ONE*, 10(5), e0125816.
- Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ros damage and regulating ros signaling. *Journal of Cell Biology*, 217(6), 1915. doi:10.1083/jcb.201708007
- Węgrzyn, E. (2013). Resource allocation between growth and endothermy allows rapid nestling development at low feeding rates in a species under high nest predation. *Journal of avian biology*, 44(4), 383.
- West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*: Oxford University Press.
- Wiedenfeld, D. A., Alberts, A. C., Angulo, A., Bennett, E. L., Byers, O., Contreras-MacBeath, T., . . . Zhang, L. (2021). Conservation resource allocation, small population resiliency, and the fallacy of conservation triage. *Conservation Biology*, 35(5), 1388. doi:<https://doi.org/10.1111/cobi.13696>
- Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1741), 20160447. doi:10.1098/rstb.2016.0447
- Winkler, D., Hallinger, K. K., Ardia, D. R., Robertson, R. J., Stutchbury, B. J., & Cohen, R. R. (2020). Tree swallow (*tachycineta bicolor*). *The Cornell Lab of Ornithology Birds of the World Version: 1.0*.
- Winkler, D. W., Wrege, P. H., Allen, P. E., Kast, T. L., Senesac, P., Wasson, M. F., & Sullivan, P. J. (2005). The natal dispersal of tree swallows in a continuous mainland environment. *Journal of Animal Ecology*, 74(6), 1080.
- Wojciechowski, M. S., Kowalczywska, A., Colominas-Ciuró, R., & Jefimow, M. (2021). Phenotypic flexibility in heat production and heat loss in response to thermal and hydric acclimation in the zebra finch, a small arid-zone passerine. *Journal of Comparative Physiology B*, 191(1), 225.
- Wolf, S. E., Sanders, T. L., Beltran, S. E., & Rosvall, K. A. (2022). The telomere regulatory gene pot1 responds to stress and predicts performance in nature: Implications for telomeres and life history evolution. *Molecular Ecology*, n/a(n/a). doi:<https://doi.org/10.1111/mec.16237>
- Wolf, S. E., Stansberry, K. R., Content, K. R., & Rosvall, K. A. (2021). A putative telomerase activator has tissue-specific effects on telomere length in a developing songbird. *Journal of Avian Biology*, 52(2). doi:<https://doi.org/10.1111/jav.02639>
- Wood, S. (2020). Mgcvm: Mixed gam computation vehicle with automatic smoothness estimation (1.8-33)[computer software]. In.
- Wood, S. N. (2006). *Generalized additive models: An introduction with r*: chapman and hall/CRC.

- Woodruff, M. J., Zimmer, C., Ardia, D. R., Vitousek, M. N., & Rosvall, K. A. (2022). Heat shock protein gene expression varies among tissues and populations in free living birds. *Ornithology*. doi:10.1093/ornithology/ukac018
- Wright, H. C., Price, J. W., Trent, J. A., Soehren, E. C., & Rush, S. A. (2019). Southward breeding expansion of tree swallows in Alabama. *Southeastern Naturalist*, 18(4), 548.
- Xie, J. J., Tang, L., Lu, L., Zhang, L. Y., Xi, L., Liu, H. C., . . . Luo, X. G. (2014). Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (*Gallus gallus*). *PLoS ONE*, 9(7). doi:ARTN e102204
10.1371/journal.pone.0102204
- Xu, Z., FitzGerald, G., Guo, Y., Jalaludin, B., & Tong, S. (2016). Impact of heatwave on mortality under different heatwave definitions: A systematic review and meta-analysis. *Environment International*, 89-90, 193. doi:<https://doi.org/10.1016/j.envint.2016.02.007>
- Zhang, Q., Han, X., Hao, X., Ma, L., Li, S., Wang, Y., & Du, W. (2018). A simulated heat wave shortens the telomere length and lifespan of a desert lizard. *Journal of thermal biology*, 72, 94.
- Zhao, H., Ke, H., Zhang, L., Zhao, Z., Lai, J., Zhou, J., . . . Li, Q. (2022). Integrated analysis about the effects of heat stress on physiological responses and energy metabolism in *Gymnocypris chilianensis*. *Science of The Total Environment*, 806, 151252. doi:<https://doi.org/10.1016/j.scitotenv.2021.151252>