

RESEARCH ARTICLE

Social hierarchy reveals thermoregulatory trade-offs in response to repeated stressors

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ABSTRACT

Coping with stressors can require substantial energetic investment, and when resources are limited, such investment can preclude simultaneous expenditure on other biological processes. Among endotherms, energetic demands of thermoregulation can also be immense, yet our understanding of whether a stress response is sufficient to induce changes in thermoregulatory investment is limited. Using the black-capped chickadee as a model species, we tested a hypothesis that stress-induced changes in surface temperature (T_s), a well-documented phenomenon across vertebrates, stem from trade-offs between thermoregulation and stress responsiveness. Because social subordination is known to constrain access to resources in this species, we predicted that T_s and dry heat loss of social subordinates, but not social dominants, would fall under stress exposure at low ambient temperatures (T_a), and rise under stress exposure at high T_a , thus permitting a reduction in total energetic expenditure toward thermoregulation. To test our predictions, we exposed four social groups of chickadees to repeated stressors and control conditions across a T_a gradient ($n=30$ days/treatment/group), whilst remotely monitoring social interactions and T_s . Supporting our hypothesis, we show that: (1) social subordinates ($n=12$), who fed less than social dominants and alone experienced stress-induced mass-loss, displayed significantly larger changes in T_s following stress exposure than social dominants ($n=8$), and (2) stress-induced changes in T_s significantly increased heat conservation at low T_a and heat dissipation at high T_a among social subordinates alone. These results suggest that chickadees adjust their thermoregulatory strategies during stress exposure when resources are limited by ecologically relevant processes.

KEY WORDS: Dominance, Stress, Thermoregulation, Birds

INTRODUCTION

The vertebrate stress response is characterised by coordinated behavioural and physiological modifications that enhance an individual's ability to cope with perceived challenges, or 'stressors'. Over the past century, research pertaining to stressor-induced physiological modifications has undergone overwhelming expansion (e.g. Cannon, 1932; Romero et al., 2009; Selye, 1950; lay literature: Sapolsky, 2004). This expansion has permitted the

identification of numerous adaptations among vertebrates that not only serve to mediate stressors acutely but also serve to minimise the potential of wear and tear acquired from long-term activation of emergency pathways (that is, to reduce allostatic load; McEwen, 1998). To ecologists, adaptations of this latter form are of particular interest as they commonly involve re-allocation of resources among biological processes (e.g. 'trade-offs') that can reveal hierarchies of investment in a species. To date, the vast majority of studies investigating stress-induced trade-offs have focused on changes to reproduction (Calisi et al., 2008; Grachev et al., 2013; Kinsey-Jones et al., 2009; Kirby et al., 2008) and immune function (Stier et al., 2009; Svensson et al., 1998), probably owing to the importance of each process in dictating individual fitness, and the relatively high costs associated with their activation and maintenance (Martin et al., 2003; Ots et al., 2001; Speakman, 2008; Vleck, 1981; but see Merlo et al., 2014). Among endothermic vertebrates, however, the energetic costs of maintaining a balanced core temperature can often overwhelm those of both reproductive and immune function (Burness et al., 2010; King and Swanson, 2013; Nord et al., 2010), and failing to maintain a balanced core temperature can be equally as detrimental to individual fitness as failing to maintain reproduction or immunity. Nevertheless, trade-offs with respect to thermoregulatory investment in the presence of stressors remain poorly understood.

Changes in body temperature in response to stressors have been widely reported across vertebrate taxa (e.g. squamates: Cabanac and Gosselin, 1993; birds: Greenacre and Lusby, 2004; fish: Rey et al., 2015; old-world primates: Parr and Hopkins, 2000; rodents: Dymond and Fewell, 1998; Yokoi, 1966) and have been documented in medical literature for nearly two thousand years (Galen, 2nd century CE; Yeo, 2005). Although the proximate mechanisms driving thermal responses to stressors have since been well characterized (e.g. redistribution of blood flow, changes in brown adipose metabolism, secretion of pyrogenic cytokines; Kataoka et al., 2014; reviewed in Oka et al., 2001), an adaptive value of this phenomenon remains contended. Indeed, whilst some studies posit that thermal fluctuations per se may endow an individual with immunological advantages during coping (Oka et al., 2001), others argue that stress-induced thermal fluctuations are merely 'spandrels' (i.e. phenotypic traits that emerge, simply, as by-products of other adaptive phenotypes; Gould and Lewontin, 1979) that emerge from the redistribution of blood flow for other adaptive purposes (e.g. haemorrhage mitigation or re-distribution of oxygen and glucose; Bartlett, 1912; Jerem et al., 2015; Jerem et al., 2018), or as artifacts of experimental design (Andreasson et al., 2019; Nord and Folkow, 2019).

Recently, we proposed that an adaptive value of stress-induced changes in body temperature may be understood when contextualised according to an individual's perceived thermoregulatory costs, and when changes in body temperature are observed at the level of an individual's surface tissues

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(Robertson et al., 2020). Specifically, we posited that changes in surface temperature (T_s) following stress exposure occur to reduce thermoregulatory costs incurred during activation of a stress response by reducing dry heat loss (i.e. non-evaporative heat loss) at temperatures below thermoneutrality, and increasing dry heat loss at temperatures above thermoneutrality. Under this hypothesis (henceforth, the ‘thermoprotective hypothesis’), stress-induced changes in T_s may therefore be viewed as products of a trade-off (similar to suggestions by Lewden et al., 2017), whereby individuals allocate energy away from metabolically expensive thermoregulatory processes (e.g. shivering and non-shivering thermogenesis in cold, and evaporative cooling in heat; costs reviewed in McKechnie et al., 2016), and toward self-preservation. Supporting the thermoprotective hypothesis, numerous studies have reported an influence of ambient temperature (T_a) on both the magnitude and direction of stress-induced thermal responses at the skin (Jerem et al., 2019; Lewden et al., 2017; Nord and Folkow, 2019; Yokoi, 1966). These studies suggest that such changes may indeed represent the presence of a trade-off between stress responsiveness and thermoregulation. Importantly, however, a critical assertion of a trade-off is that its emergence is dependent upon a limitation of energy or resources with which one can invest (Stearns, 1992; reviewed in Breuner and Berk, 2019). Whilst some studies have provided strong suggestive evidence of a link between resource access and baseline T_s in birds (Jerem et al., 2018; Winder et al., 2020), empirical studies testing the effect of resource constraint on T_s responses to stress exposure are lacking (but see suggestive evidence in Winder et al., 2020). Such studies are imperative to further our understanding of whether stress responsiveness may influence investment in thermoregulation among vertebrates.

Using the black-capped chickadee (*Poecile atricapillus* Linnaeus 1766; a social, temperate endotherm) as a model species, we sought to understand whether stress-induced changes in T_s emerge from trade-offs between thermoregulation and stress responsiveness – a yet-untested assumption of the thermoprotective hypothesis. In black-capped chickadee social groups, social subordination is widely thought to constrain an individual’s access to resources (i.e. access to supplemental food, and mass; Ficken et al., 1990; Glase, 1973; Smith, 1991; shown here), and as such may represent an ecologically relevant mediator of trade-off expression. We therefore asked: (1) whether an individual’s social status dictates the magnitude of their T_s response to a stressor, and (2) whether the direction of an individual’s T_s response to stress exposure enhances conservation of energy by decreasing dry heat loss in the cold, and increasing dry heat dissipation in the warmth. To answer these questions, we leveraged data from a previous experiment wherein we exposed social groups of black-capped chickadees to randomised and repeated stressors across a naturally occurring T_a gradient, whilst monitoring feeding behaviour, T_s and total rate of dry heat loss (q_{tot}) by digital video and infrared thermography (Robertson et al., 2020). Under the thermoprotective hypothesis, we predicted that the T_s and q_{tot} of socially subordinate individuals (henceforth, ‘subordinates’) would be more sensitive to stress exposure than those of socially dominant individuals (henceforth, ‘dominants’) across an T_a spectrum, owing to their comparatively limited resource access. More specifically, we predicted that subordinates (who typically have lower access to available food, and lower fat stores) would experience a greater fall in T_s and q_{tot} at low T_a , and a greater rise in T_s and q_{tot} at high T_a than dominants when exposed to repeated stressors. We predicted this because lower access to resources in subordinate individuals would preclude simultaneous investment in metabolic heating or cooling (i.e.

shivering and non-shivering thermogenesis, and evaporative cooling) and self-protection.

To our knowledge, this is the first study to test whether thermal responses to stress exposure are influenced by resource access across naturally occurring T_a below, at, and above a species’ thermoneutral zone. Furthermore, it is also one of few to explore the presence of a trade-off under stress exposure with respect to ecologically relevant resource constraints (e.g. those induced by social hierarchies; Aghajani et al., 2013; Proctor et al., 2010). Together, we argue that this is the first study to interrogate a trade-off between stress responsiveness and thermoregulation that is implicit in the thermoprotective hypothesis.

MATERIALS AND METHODS

All protocols used for animal capture, handling and experimental manipulation were approved by the Government of Canada (Environment and Climate Change Canada; permit #10756E) and by the Trent University Animal Care Committee (AUP #24614).

Capture and sampling of black-capped chickadees

Black-capped chickadees used for experimentation ($n=20$) were captured within a 100 km² region of Southern Ontario (Canada), during March and April of 2018. We divided capture efforts across both urban and rural locations ($n=3$ sites per category) to control for possible effects of urbanisation on stress-induced changes in T_s alone. Urban capture locations included the cities of Cambridge (43.379°N, 80.353°W), Guelph (43.330°N, 80.150°W) and Brantford (43.135°N, 80.344°W), whilst rural capture locations included the greater townships of Erin and Corwhin (43.762°N, 80.153°W and 43.509°N, 80.090°W, respectively), and the Ruthven Park National Historic Site (42.980°N, 79.875°W). Our final sample population included 10 individuals captured from urban locations ($n=5$ females, $n=5$ males), and 10 individuals captured from rural locations ($n=5$ females, $n=5$ males).

All black-capped chickadees were captured using modified Potter traps that were baited with sunflower seeds on the morning of capture. Immediately following capture, individuals were blood sampled for genetic sexing by brachial venipuncture (approximately 50 µl), then assigned a unique combination of one government-issued stainless steel leg band (size 0; Environment and Climate Change Canada; Bird Banding Office) and two coloured leg bands (Darvic, 2.3 mm interior diameter; Avinet, Portland, ME, USA) for individual identification. Birds were then weighed and measured (mass: nearest 0.1 g using a digital platform scale; left tarsus and flattened wing-chord; nearest 1 mm using analogue callipers), and secured in transportation cages (30.0 cm×30.0 cm×15.0 cm; 1×w×h) for translocation to long-term holding facilities at the Ruthven Park National Historic Site, Cayuga, ON, Canada (<90 km; 2 h travel by vehicle). Erythrocytes were isolated from blood samples by centrifugation in the field (12,000 rpm), preserved in Queen’s lysis buffer (Seutin et al., 1991), then stored at 4°C until required for sex determination (see ‘Sex determination’, below).

Captive maintenance

Housing of black-capped chickadees is described elsewhere (Robertson et al., 2020). Briefly, all birds were randomly allocated to one of four mixed-sex and visually isolated flight enclosures ($n=5$ per flight enclosure; 1.83 m×1.22 m×2.44 m; 1×w×h), each supplied with a roosting box (60 cm×20×20 cm; 1×w×h), one roosting tree (white cedar, *Thuja occidentalis*; 1.0 m), and two perching branches (approximately 80 cm in length). Enclosures were fitted with one feeding platform (0.4 m²) placed

at approximately 1.2 m in height, that could be provisioned from behind an opaque barrier to ensure that birds were blind to the presence of experimenters. Additionally, a small, water-tight camera box perforated with two small holes (30 mm in diameter) was secured to an outside wall of each flight enclosure, and positioned parallel to each feeding platform to permit filming of individuals through the perforations during feeding (described in ‘Thermographic imaging, digital filming and environmental data collection’, below). Camera boxes were not visible to our study individuals and were solely accessed from the exterior of the enclosures.

Black-capped chickadees were acclimated to flight enclosures for a minimum of 2 weeks before experimentation. During both acclimation and experimentation periods, all birds were fed a mixture of mealworms (*Tenebrio molitor*), crickets (*Acheta domesticus*), sunflower seeds, safflower seeds, shelled peanuts, apple pieces, boiled egg and Mazuri™ (St Louis, MO, USA) Small Bird Maintenance diet *ad libitum*, and were supplied with *ad libitum* fresh water each day. Minimum and maximum T_a observed during our experiment were 2.5 and 38.5°C, respectively, and minimum and maximum daylight hours ranged from approximately 13.7 h (sunrise 06:29 h, sunset 20:08 h) to 15.4 h (sunrise 05:38 h, sunset 21:02 h).

Experimental stress exposure

To experimentally induce a stress response among black-capped chickadees, we employed a rotational stress exposure protocol similar to that used in Rich and Romero (2005). A rotational approach to stress exposure was chosen to circumvent habituation to each individual stressor across the duration of our experiment. To increase statistical power, we used a repeated sampling approach whereby all individuals received both a control and a stress exposure treatment, with each treatment persisting for 30 days and being separated by a rest period of 2 days (total experimental duration of 62 days). To control for possible effects of treatment order, and to control for the effects of seasonal changes in T_a and day length that may influence stress physiology within our sample population, individuals were divided into two groups of 10 individuals ($n=2$ flight enclosures per group; one west facing, and one east facing per grouping), with one group receiving a control treatment followed by a stress-exposure treatment, and the other group simultaneously receiving a stress-exposure treatment followed by a control treatment.

In stress-exposure treatments, individuals were exposed to five or six randomly selected, passive stressors each day, with each exposure persisting for 20 min and being separated from previous and subsequent stressors by 1 h. Here, the total number of stressor exposures applied in a given day (i.e. five or six) was also randomly chosen each day. Possible stressors included: (1) presence of an experimenter within the flight enclosure of target individuals, (2) capture and restraint in an opaque, fabric bag, (3) presence of a mock predator (adult Cooper’s hawk, *Accipiter cooperii*), (4) presence of a taxidermised conspecific, mounted to the feeding platform of target individuals, (5) presence of a novel object (garden gnome) placed near the feeding platform of target individuals, and (6) covering of the flight enclosure with an opaque fabric sheet. Auditory stressors were not used in this experiment to avoid evocation of stress responses in study individuals housed in nearby flight enclosures. The effectiveness of stress-exposure treatments was confirmed by monitoring behavioural responses to each passive stressor (e.g. elicitation of alarm calls, elicitation of avoidance behaviour, and reduction in feeding rate of stress-exposed

individuals relative to controls; see Results); endocrine responses to stress-exposure treatments were not assessed to avoid interference of control treatments from blood sampling. Importantly, we recognise that our flight enclosures were not auditorily isolated and elicitation of alarm calls from individuals within stress-exposure treatments was likely to be detected by those in control treatments. Consequently, analyses of differences in T_s between treatment times are likely to be conservative.

In control treatments, individuals were maintained according to acclimation conditions, and were not handled or exposed to experimenters. All black-capped chickadees were returned to their location of capture and released upon completion of our experiment.

Thermographic imaging, digital filming and environmental data collection

To monitor the T_s of black-capped chickadees during experimental treatments, we passively imaged individuals during feeding using a remotely operated infrared thermographic camera (VueProR™, FLIR, Wilsonville, OR, USA; 13 mm lens, 336×256 resolution) that was placed in a water-tight camera box and oriented toward a focal feeding platform at a distance of 0.5 m (camera boxes are described in ‘Captive maintenance’, above). Thermographic filming was conducted at a frequency of 1 image per second for a minimum of 1 h per enclosure, per day. Because time of day is likely to influence body temperature in chickadees (Reinertsen and Haftorn, 1984), we rotated thermographic filming among flight enclosures (cardinally clockwise) within each day, and initiated filming at a randomly determined flight enclosure each morning to control for the effect of time alone on T_s readings. Identity of individuals within thermographic images was determined from digital video that was captured in parallel to thermographic images (whereby a digital video camera was rotated alongside our thermographic camera; digital camera: Action Cam™, Sony, Toronto, ON, Canada). All thermographic images and digital video used in this study were captured between 08:00 h and 16:00 h across a total of 60 days. In total, data from 6431 thermal images were used for this study, with 3034 images captured during control treatments (subordinates: $n=1494$ images, dominants: $n=1540$ images), and 3397 images during stress exposure (subordinates: $n=1943$ images, dominants: $n=1454$ images).

Both T_a and relative humidity are known to influence estimates of an object’s T_s by infrared thermography (reviewed in Minkina and Dudzik, 2009; Tattersall, 2016). We therefore collected T_a and relative humidity measurements across the duration of experimentation. T_a measurements were recorded within flight enclosures during thermographic filming intervals using a ThermoChron iButton™ (1 reading/5 min at a resolution of 0.5°C; Maxim Integrated, DS1922L-F5; San Jose, CA, USA) placed in the shade, whilst relative humidity readings were collected from a governmental climate repository (Department of Environment and Climate Change Canada; <https://climate.weather.gc.ca/>; Hamilton A; 22 km from the site of experimentation). Relative humidity readings were collected at a frequency of 1 reading/hour (the highest resolution available).

Social status determination

Social status of our study individuals was estimated from enumerated presence and absence data derived from digital video observations made in parallel to thermographic filming (enumeration methods described in the Appendix). Here, individual social status was assessed per flight enclosure, using a similar approach to that recommended for our study species by

Evans et al. (2018). Specifically, presence and absence data at feeding platforms were used to detect displacement events (where the arrival of individual A resulted in the departure of individual B), waiting events (where the arrival of individual B was contingent upon the departure of individual A), and simultaneous feeding events (where individuals A and B fed simultaneously) among chickadees, across the duration of our experiment ($n=60$ days; criteria for each interaction type are described in the Appendix). Individuals involved in displacement or waiting events were then assigned a 'win' or 'loss' according to whether they received priority access to the feeding platform, whilst those involved in non-agonistic interactions were assigned a 'draw'. Total wins, losses and draws per individual were then used to calculate an index of relative wins in social interactions, and this index was used to infer social status values of chickadees within each flight enclosure. For this study, we chose to use a randomised Elo rating (Elo, 1978) as our index of relative wins because it is thought to outperform other widely used status indices (e.g. David's Score, ADAGIO, IS&S) when predicting social status in the presence of shallow dominance hierarchies (Sánchez-Tójar et al., 2018).

To calculate randomised Elo ratings per individual, we randomly sampled 20 days of observation data, then used these data to calculate final, non-randomised Elo ratings per individual in the R package 'EloRating' (<https://CRAN.R-project.org/package=EloRating>). This process was iterated 1000 times and mean, final Elo ratings $\pm 95\%$ confidence intervals (CIs) were calculated per individual, per flight enclosure, across iterations. To ensure that our Elo rating estimates were conservative, we assumed that displacement events held greater importance in defining an individual's Elo rating than waiting events (sensitivity score, $K=100$ and 50 for displacement and waiting events, respectively), given that feeding order and time may be randomly determined. Black-capped chickadees within each flight enclosure were then ranked in descending order according to their mean Elo rating (rank 1=most dominant, rank 5=least dominant), then categorised as either socially dominant or socially subordinate. Here, two individuals with the highest mean Elo ratings within each enclosure were considered socially dominant ($n=2$ per enclosure; $n=8$ total; Fig. 1), whilst the remaining individuals were considered socially subordinate ($n=3$ per enclosure; $n=12$ total; Fig. 1). Partitioning of individuals in this way was chosen to balance sample size between status groups whilst minimising the number of individuals experiencing regular top-down antagonism in socially dominant categories.

To assess our confidence in social status estimates, we calculated a 'status-stability index' (SSI) per individual, which describes the proportion of final status estimates (that is, those assumed upon completion of each random sampling of observation dates) that were found to be equivalent to an individual's modal social status estimate [similar to van Hooff and Wensing's (1987) directional consistency index, but expanded for application to randomised data]. SSI per individual was calculated using the following equation:

$$SSI_{ij} = \left(\sum_{i=1:n_{it}} |S_{ij} - \text{modal}(S_j)| \right) \cdot n_{it}^{-1}, \quad (1)$$

where S represents the estimated social status of an individual j according to their Elo rating that was calculated following a randomised iteration i , $\text{modal}(S_j)$ represents the modal social status of individual j across all iterations i (consequently, the final estimated social status for an individual j), n_{it} represents the total number of randomised iterations, and n_{birds} represents the total number of individuals for which social statuses were estimated.

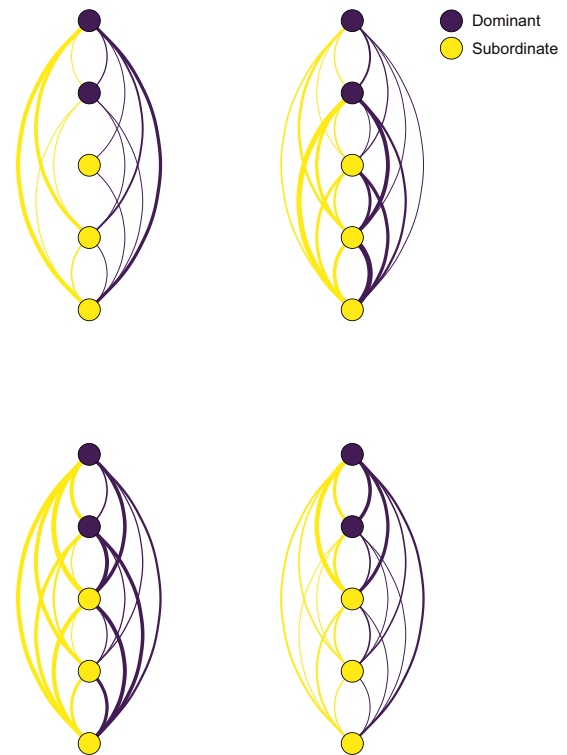


Fig. 1. Linear social hierarchies of black-capped chickadees ($n=20$) across four flight enclosures ($n=5$ birds per enclosure) and 60 days of observation. Purple circles represent individuals categorised as dominant, whilst yellow circles represent those categorised as subordinate; linear order of circles represents the estimated order within a dominance hierarchy (with the most socially dominant individual at the top, and least socially dominant individual at the bottom, as estimated using randomised Elo ratings). Lines represent agonistic interactions between individuals (i.e. supplantations and waiting events), with purple lines representing top-down interactions (dominant to subordinate), and yellow lines representing bottom-up interactions (subordinate to dominant). Line width is proportional to the number of interactions between individuals in a dyad.

Calculation of T_s and heat transfer from thermographic images

T_s measurements [maximum temperature of the periorbital ('eye') region] of black-capped chickadees were derived from thermal images in R (approximately 230 pixels, or 0.3% of the image region; <http://www.R-project.org/>) according to previously described methods (Robertson et al., 2020). Emissivity of black-capped chickadee integument was estimated as 0.95, according to Best and Fowler (1981). We chose to assess T_s at the eye region alone because it is readily imaged during feeding events, it contains exposed integument that is uninsulated by keratinous tissues (i.e. leg scales or feathers; discussed by Jerem et al., 2018), and it has previously been shown to be thermally responsive to stress exposure (e.g. Jerem et al., 2015; Robertson et al., 2020). Additionally, we chose to monitor the maximum eye region temperature rather than the mean eye region temperature because it is thought to be less vulnerable to measurement error, and more consistent across image angles (Jerem et al., 2015, 2018). Similar to Robertson et al. (2020), T_s measurements were only extracted from images where identity of the focal individual could be determined by video observations, and

when the focal individual was not in motion (to avoid underestimation of T_s ; discussed in Tattersall, 2016).

We quantified the rate of heat transfer (mW) from study individuals using T_s measurements (derived from thermographic images) and equations described by McCafferty et al. (2011) and Nord and Nilsson (2019). Heat transfer rates were therefore limited to those experienced at the eye region alone. Because our chickadees were housed in semi-protective flight enclosures that provided shelter from wind, and because conductive heat transfer from the eye region to a medium other than air was unlikely, we assumed that q_{tot} was equal to the sum of convective and radiative heat transfer alone (q_{conv} and q_{rad} , respectively), at a given second. Estimates of the thermal conductivity of air ($\text{W m}^{-1} \text{ } ^\circ\text{C}^{-1}$), the kinematic viscosity of air ($\text{m}^2 \text{ s}^{-1}$; at an assumed atmospheric pressure of 101.325 kPa), and the thermal expansion coefficient of air ($1/k$) were allowed to vary according to the T_a experienced at the time of image capture, and kinematic viscosity and air conductivity calculations were conducted using the R packages ‘bigleaf’ and ‘Thermimage’, respectively (Knauer et al., 2018; <https://CRAN.R-project.org/package=Thermimage>). Eye region surfaces were treated as planar, ovoid structures with a vertical diameter of 1.0 cm and a horizontal diameter of 1.1 cm (surface area = 0.864 cm^2). All heat transfer calculations were conducted in R and final q_{tot} values were multiplied by 2 to represent total heat transfer across both eye regions of an individual.

Sex determination

Sex of all experimental individuals was determined according to methods described by Griffiths et al. (1996) and Fridolfsson and Ellegren (1999). Specifically, we isolated whole genomic DNA from erythrocyte-lysis samples by phenol:chloroform:isoamyl (25:24:1) extraction and 2-propanol precipitation, then amplified intron 16 of the chromohelicase DNA-binding gene (*CHD* gene) by PCR. Intron 16 of the *CHD* gene differs in base-pair length between copies located on the W and Z chromosomes. The sex of individuals was therefore determined by size-separation of PCR amplicons on 3% agarose gels (120 V).

Statistical analyses

All statistical analyses were conducted in R, and α levels were set to 0.05. All generalised additive mixed-effects models (GAMMs) were fitted with restricted maximum likelihood (REML) in the R package ‘mgcv’ (Wood, 2011), as recommended by Simpson (2018).

Effect of social status on stress-induced changes in T_s

We predicted that subordinate individuals would elicit a larger change in T_s following stress exposure than dominant individuals. To test this prediction, we used a GAMM with maximum eye region temperature (mean temperature for a given hour of observation; $n=1027$ observations across $n=60$ days) as the response variable (Gaussian distributed). Use of an additive model (in place of a linear model) was chosen to best capture the non-linear relationship between T_s and T_a that has been previously reported in black-capped chickadees (Reinertsen and Haftorn, 1984). Treatment (binomial factor; ‘control’ or ‘stress-induced’), social status (binomial factor; ‘dominant’ or ‘subordinate’) and sex (binomial factor) were each included as linear predictors in our model, and an interaction between treatment and social status was also included to account for differential responses to stress exposure across social hierarchies that may occur independently of T_a . An effect of T_a on the T_s of black-capped chickadees was tested by inclusion of mean hourly

temperature ($^\circ\text{C}$) as a cubic regression spline, with four knots to capture non-linearity and circumvent model over-fit. To test whether the effect of treatment on T_s varied across T_a , we included mean hourly T_a and treatment as a tensor product predictor, with treatment being represented as a smooth factor and mean hourly T_a again being represented as a cubic regression spline with four knots. Importantly, to test the effect of social status on the magnitude of an individual’s thermal response to stress across T_a , a linear interaction between social status, and our hourly T_a and treatment tensor product was included with first-order derivative penalisation.

Because black-capped chickadees may display diel changes in body temperature that occur independently of T_a (Robertson et al., 2020), we sought to include time of day (hour) as a predictor in our model; however, we also recognised that the effect of time of day was likely to differ according to flight enclosure orientation (i.e. west or east facing), owing to differences in exposure to solar radiation at each hour. For this reason, a tensor product between time of day and flight enclosure orientation (i.e. ‘east’ or ‘west’; $n=2$ per cardinal direction) was included as a predictor in our model, and because stress exposure was previously reported to shift the relationship between time of day and T_s , a linear interaction between treatment and our time of day by enclosure tensor product was also included (with first-order derivative penalisation, and with spline conditions as described above). Here, time of day was modelled using a penalised cubic regression spline with four knots (cubic shrinkage spline to minimise function size), while enclosure orientation was modelled as a smooth factor. To capture variance explained by date of imaging, individual identity and flight enclosure, random intercepts were included for each factor. Temporal autocorrelation was not detected within individuals in our model ($\rho=-0.151$). Furthermore, although previous studies have reported correlations between sex and social status in black-capped chickadees (e.g. Glase, 1973; Desrochers et al., 1988), inclusion of both factors as parametric predictors in our model did not contribute to variance inflation (variance inflation factors, $\text{VIFs}<2$ for all parametric predictors; as estimated using the R package ‘car’; Fox and Weisberg, 2019).

Changes in T_s are likely to yield the greatest energetic consequences at T_a above or below thermoneutrality. We therefore tested whether the T_s of dominant and subordinate individuals differed between control and stress-exposure treatments at T_a below, at and above the thermoneutral zone for black-capped chickadees ($14\text{--}30^\circ\text{C}$; lower limit; Grossman and West, 1977; upper limit; Rising and Hudson, 1974). To do so, we conducted planned comparisons between treatment types within each temperature zone (i.e. sub-thermoneutral, thermoneutral, supra-thermoneutral; $n=3$ contrasts), and for each level of social status in the R package ‘emmeans’ (<https://CRAN.R-project.org/package=emmeans>). Degrees of freedom for each planned comparison were estimated using Kenward–Roger approximations. For this analysis, a broad thermoneutral zone was assumed because our experiment spanned from late winter to early summer, during which the thermoneutral zone of black-capped chickadees is thought to change (Rising and Hudson, 1974).

Differences in heat transfer across social status

To understand whether differences in the magnitude of stress-induced changes in T_s among individuals impact energy conservation, we asked: (1) whether stress exposure influenced q_{tot} among individuals, and (2) whether an effect of stress exposure on q_{tot} was conditional upon the social status of an individual.

Because heat transfer from an object is proportional to its T_s ($q_{\text{tot}} \propto T_s$), we modelled the relationship between q_{tot} , social status, treatment and T_a using the same statistical approach as that used for modelling T_s in our study individuals (see 'Effect of social status on stress-induced changes in T_s ', above; all predictors remain equivalent). Here, however, maximum eye region temperature was replaced with q_{tot} as the Gaussian distributed response variable ($n=1027$ observations across $n=60$ days). Again, inclusion of both sex and social status as parametric predictors did not contribute to variance inflation ($\text{VIFs} < 2$ for each predictor).

RESULTS

Social hierarchies are stable in black-capped chickadees

Throughout our experiment, we detected 1006 social interactions between individual chickadees (mean \pm s.d. = 50.300 ± 23.414 per individual). Estimates of social status derived from the outcomes of social interactions were stable when calculated across randomly sampled days of observation (SSI mean \pm s.d. = $78.020 \pm 15.360\%$; Fig. 1), and stability did not significantly differ among flight enclosures ($F=0.122$, d.f.=3, $P>0.9$). Stability indices (SSIs) exceeded 50% across all individuals (range 50.5–100%). Interestingly, social dominance did not appear to be dependent on sex in our study; rather, males and females were equally represented in dominant and subordinate positions (dominant: $n=4$ males, $n=4$ females; subordinate: $n=6$ males, $n=6$ females). Unsurprisingly, subordinates accessed supplemental food less frequently than dominants (see Appendix, Fig. A1) and alone experienced a loss of mass during stress-exposure treatments (see Appendix, Fig. A2) despite food being provided *ad libitum* throughout our experiment. Together, these results support our assertion of resource restriction among subordinate individuals in our sample population.

Effects of stress exposure on T_s differ according to social status

Maximum eye temperature was positively correlated with T_a in our study animals ($P<0.001$; Table 1, Fig. 2). As predicted by the

thermoprotective hypothesis, this relationship was significantly influenced by treatment in subordinate individuals (social status: tensor product, henceforth \otimes , of treatment and T_a : $P=0.026$; Table 1, Fig. 2), but not dominant individuals (treatment $\otimes T_a$: $P=0.285$; Table 1, Fig. 2). Specifically, at T_a below thermoneutrality ($<14^\circ\text{C}$), T_s of subordinates was significantly lower during stress-exposure treatments than during control treatments (mean \pm s.e.m. difference, $\Delta = -1.579 \pm 0.508^\circ\text{C}$, $t_{1006}=3.109$, $P=0.002$; Fig. 3), whilst that of dominants did not significantly differ between treatment types ($\Delta = -0.412 \pm 0.432^\circ\text{C}$, $t_{1006}=0.955$, $P=0.340$; Fig. 3). Similar trends were observed at T_a above thermoneutrality ($>30^\circ\text{C}$), with T_s of subordinates, but not dominants, significantly rising under stress-exposure treatment (mean \pm s.e.m.; subordinates: $\Delta = 1.220 \pm 0.501^\circ\text{C}$, $t_{1006}=2.436$, $P=0.015$; dominants: $\Delta = 0.636 \pm 0.502^\circ\text{C}$, $t_{1006}=1.267$, $P=0.205$; Fig. 3). At T_a within the thermoneutral zone, however, the T_s of neither subordinate nor dominant individuals significantly differed between treatment types (subordinates: $t_{1006}=0.444$, $P=0.657$; dominants: $t_{1006}=0.098$, $P>0.9$; Fig. 3).

Interestingly, neither treatment nor social status independently explained T_s in our study animals (treatment: $P=0.706$; social status: $P=0.795$; Table 1); an interaction between each factor was also insufficient to explain T_s profiles ($P=0.726$; Table 1). The combined effects of time of day and flight enclosure orientation (i.e. east or west facing; $n=2$ per cardinal direction) were, however, significantly correlated with T_s (time of day \otimes enclosure orientation: $P<0.001$; Table 1), and this diel rhythm differed according to treatment type (treatment: time of day \otimes enclosure orientation: $P<0.001$; Table 1). A main effect of sex on T_s was not significant ($P=0.895$; Table 1).

Social status influences the effects of stress exposure on dry heat transfer

Similar to our results regarding T_s , total dry heat transfer (q_{tot}) was significantly correlated with T_a ($P<0.001$; Table 2). As predicted by the thermoprotective hypothesis, this relationship between T_a and q_{tot} was significantly influenced by stress exposure in subordinates

Table 1. Results of a GAMM testing the influence of social status on the relationship between ambient temperature and surface temperature of black-capped chickadees ($n=20$) across control and stress-exposed conditions

Predictors	Coefficient	s.e.m.	d.f./e.d.f.	t/F	P
Linear predictors					
Intercept	33.943	0.541	1	62.735	<0.001*
Status	−0.047	0.181	1	−0.260	0.795
Sex	0.036	0.273	1	0.132	0.895
Stress exposure	0.119	0.315	1	0.377	0.706
Stress exposure: Status	0.044	0.125	1	0.351	0.726
Smooth predictors					
T_a	6.980	0.458	2.447	157.261	<0.001*
$T_a \otimes$ Stress exposure	−0.405	0.600	2.618	0.802	0.285
Status: $T_a \otimes$ Stress exposure	−0.217	0.480	2.626	1.154	0.026*
Time of day \otimes Enclosure orientation	−0.018	0.581	5.429	7.039	<0.001*
Stress exposure: Time of day \otimes Enclosure orientation	−0.103	0.542	3.287	4.613	<0.001*
Random effects					
Predictor	Variance				
Bird identity	0.471				
Date of photo	0.818				
Enclosure identity	0.818				

Mean surface temperature (T_s) of chickadees ($n=8$ dominant; $n=12$ subordinate) was measured across 60 days (30 days per treatment type), and averaged by hour; results of a generalised additive mixed-effects model (GAMM) are shown. Coefficient estimates (β for linear predictors, and mean β for smooth predictors) and standard errors around coefficient estimates (s.e.m.) are presented for each fixed-effect predictor. Degrees of freedom (d.f.) are reported for linear predictors, and estimated degrees of freedom (e.d.f.) for smooth predictors. t -values are reported for linear predictors and F -values for smooth predictors. Asterisks signify a significant effect at $\alpha=0.05$, and \otimes represents a tensor product. Social dominants: $n=458$ measurements; social subordinates: $n=569$ measurements. This model explained 74.941% of deviance in T_s data.

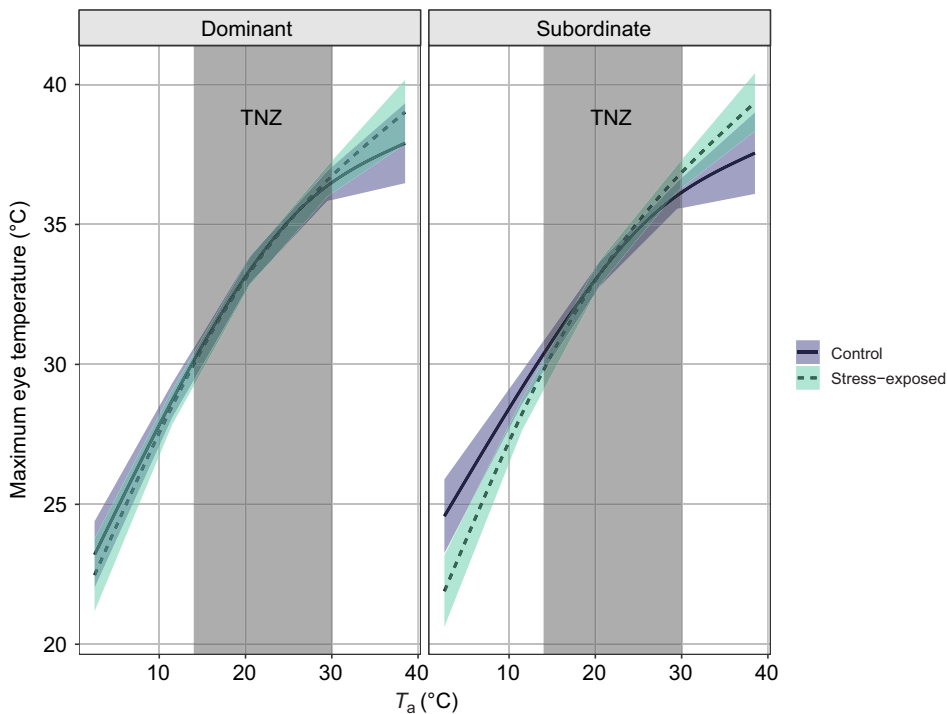


Fig. 2. Effects of repeated stress exposure on eye region temperature of dominant and subordinate black-capped chickadees across ambient temperature. Eye temperature measurements (mean, hourly; representing surface temperature, T_s) were calculated from infra-red thermographic images that were captured from 20 black-capped chickadees across 60 days ($n=1027$ images, $n=8$ dominant, $n=12$ subordinate). Solid and dashed lines represent mean eye region temperature of individuals under control and stress-exposure treatments, respectively (marginal means, calculated from a generalised additive mixed-effects model). Stress exposure significantly influenced the relationship between ambient temperature (T_a) and eye region temperature in social subordinates alone ($P=0.026$). Purple and green bands represent 95% confidence intervals (CIs) around mean estimates for control and stress-exposure treatments, respectively. Grey rectangles represent the estimate thermoneutral zone (TNZ) of black-capped chickadees (14–30°C).

alone (treatment $\otimes T_a$: $P>0.9$; status: treatment $\otimes T_a$: $P=0.004$; Table 2, Fig. 4). At T_a below thermoneutrality, q_{tot} of subordinates significantly fell during stress-exposure treatments (mean \pm s.e.m. $\Delta = -4.619 \pm 1.450$ mW, $t_{1005} = 3.190$, $P=0.002$; Fig. 4); a similar fall was not observed among dominants ($\Delta = -0.023 \pm 1.220$ mW, $t_{1005} = 0.019$, $P=0.985$; Fig. 4).

Interestingly, at T_a above thermoneutrality, stress exposure did not significantly influence q_{tot} in dominant or subordinate individuals (subordinates: $t_{1005} = 1.650$, $P=0.099$; dominants: $t_{1005} = 0.014$, $P>0.9$; Fig. 4); however, our analyses did detect a trend of q_{tot} increasing in response to stress-exposure treatment among subordinates alone ($P<0.1$). Neither dominants nor

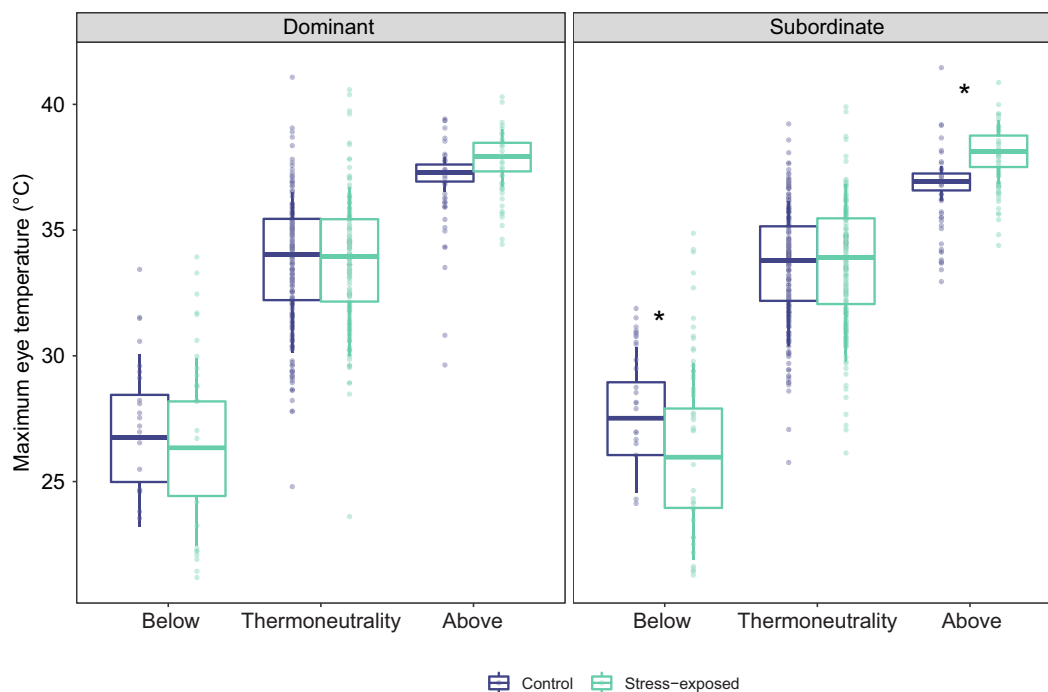


Fig. 3. Distribution of eye region temperature measurements from dominant and subordinate chickadees at T_a below, at and above estimated thermoneutrality. Eye region temperature was calculated from infra-red thermographic images captured across 60 days ($n=1027$ images, $n=20$ individuals, $n=8$ dominant, $n=12$ subordinate). Lower and upper ranges of boxes represent the 1st and 3rd quartiles, respectively, whilst whiskers represent $\pm 1.58 \times$ the interquartile range. Purple and green dots represent raw eye region temperature measurements for control and stress-exposure treatments, respectively. Asterisks represent significant differences between treatments within the different T_a ($P<0.05$); $\alpha=0.05$.

Table 2. Results of a GAMM testing the influence of social status on total dry heat loss across the eye region of black-capped chickadees ($n=20$) across control and stress-exposed conditions

Predictors	Coefficient	s.e.m.	d.f./e.d.f.	t/F	P
Linear predictors					
Intercept	26.846	1.492	1	17.993	<0.001*
Status	-0.127	0.488	1	-0.259	0.796
Sex	0.091	0.737	1	0.124	0.901
Stress exposure	0.230	0.912	1	0.252	0.801
Stress exposure: Status	0.104	0.345	1	0.302	0.763
Smooth predictors					
T_a	-19.600	1.310	2.261	103.745	<0.001*
$T_a \otimes$ Stress exposure	0.000	1.800	3.000	<0.001	1.000
Status: $T_a \otimes$ Stress exposure	-0.710	1.470	3.051	1.883	0.004*
Time of day \otimes Enclosure orientation	-0.047	1.590	5.454	7.039	<0.001*
Stress exposure: Time of day \otimes Enclosure orientation	-0.273	1.550	3.410	5.283	<0.001*
Random effects					
Predictor	Variance				
Bird identity	1.258				
Date of photo	2.223				
Enclosure identity	2.376				

Total dry heat loss (q_{tot}) was measured across the eye region of chickadees ($n=8$ dominant; $n=12$ subordinate) across 60 days (30 days per treatment type) and represent means per hour. Coefficient estimates (β for linear predictors, and mean β for smooth predictors) and standard errors around coefficient estimates (s.e.m.) are presented for each fixed-effect predictor. Degrees of freedom (d.f.) are reported for linear predictors, and estimated degrees of freedom (e.d.f.) are reported for smooth predictors. t -values are reported for linear predictors and F -values for smooth predictors. Asterisks signify a significant effect at $\alpha=0.05$, and \otimes represents a tensor product. Social dominants: $n=458$ measurements; social subordinates: $n=569$ measurements. This model explained 84.870% of deviance in T_s data.

subordinates displayed significant differences in q_{tot} at thermoneutrality (subordinates: $t_{1005}=0.420$, $P=0.675$; dominants: $t_{1005}=0.039$, $P>0.9$; Fig. 4).

We did not detect a significant main effect of treatment ($P=0.801$; Table 2), social status ($P=0.796$; Table 2) or sex ($P=0.901$; Table 2) on q_{tot} from the eye region, paralleling our

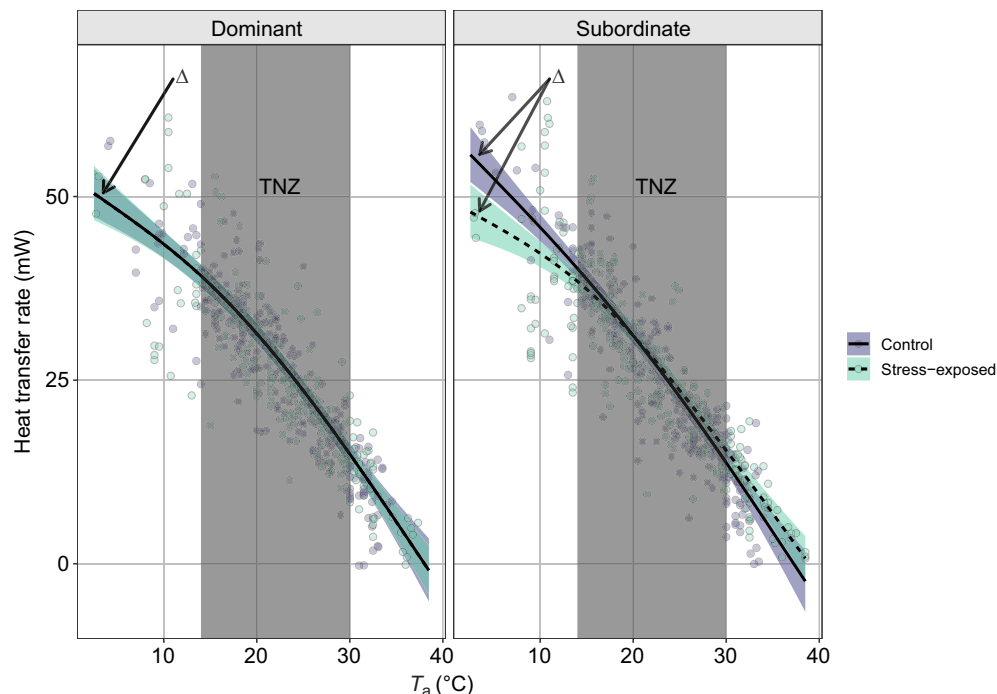


Fig. 4. Dry heat transfer across T_a in black-capped chickadees, as influenced by stress exposure and social status. Mean dry heat transfer (q_{tot} ; marginal mean) across the eye region was calculated for dominant and subordinate individuals at naturally occurring T_a , and during stress-exposure and control treatments from a generalised additive mixed-effects model ($n=8$ dominant, $n=12$ subordinate, $n=60$ days, $n=1027$ observations). Dashed lines represent mean trends under control treatments and solid lines represent mean trends under stress-exposure treatments; purple and green bands represent 95% CIs around control and stress-exposure treatment trends, respectively. Δ denotes curves between which comparisons were tested. Grey rectangles represent the estimated TNZ of black-capped chickadees (14–30°C). Purple and green dots represent raw heat transfer measurements for control and stress-exposure treatments, respectively. Among social dominants, the relationship between T_a and dry heat transfer did not significantly differ between control and stress-exposure treatments ($P=0.112$; $\alpha=0.05$). Contrastingly, social subordinates decreased dry heat loss at low T_a and increased dry heat loss at high T_a under stress exposure, when compared with controls ($P<0.001$; $\alpha=0.05$).

results regarding T_s . Furthermore, a linear interaction between social status and treatment on q_{tot} (irrespective of T_a) was not detected ($P=0.763$; Table 2). Similar to our analysis of T_s , the combined effects of time of day and enclosure orientation significantly predicted q_{tot} (time of day \otimes enclosure orientation; $P<0.001$; Table 2), and this relationship was influenced by treatment type (treatment: time of day \otimes enclosure orientation: $P<0.001$; Table 2).

DISCUSSION

T_s responses to stress exposure represent a trade-off

Stress-induced changes in T_s have previously been viewed as evolutionary ‘spandrels’ that emerge as a by-product of experimental design, or vasomotor responses that serve alternative functional purposes (e.g. Andreasson et al., 2019; Jerem et al., 2015). Here, however, we provide evidence that thermal responses to stress exposure may emerge from a trade-off between thermoregulation and stress responsiveness, whereby individuals that are resource limited (in our experiment, subordinate individuals; resource limitation supported in Appendix Figs A1 and A2) seek to reduce their investment in thermoregulation when confronted with perceived stressors (the thermoprotective hypothesis; as alluded to by Jerem et al., 2018). Indeed, our results show that during exposure to repeated stressors, subordinates experienced a greater reduction in T_s at low T_a (i.e. those below thermoneutrality), and a greater increase in T_s at high T_a (i.e. those above thermoneutrality) than dominants (Figs 2 and 3; our first prediction) when compared with subordinates under control conditions. Furthermore, this exacerbated T_s response to stressors appeared to endow subordinates with a larger decrease in heat loss (a reduced q_{tot}), and larger increase in heat dissipation (an increased q_{tot} , albeit non-significant) during stress exposure than dominants in cold and warm environments, respectively (Fig. 4; our second prediction). At T_a below thermoneutrality, for example, subordinates reduced their q_{tot} across the eye region by an average of 4.62 mW during stress exposure (Results and Appendix Fig. A1; approximately 12% of maximal dry heat transfer; 54.4 mW). Such reductions in q_{tot} are substantially larger than those experienced by dominants at equivalent T_a (−0.02 mW; Results and Appendix Fig. A1). A similar disparity in q_{tot} between dominants and subordinates was observed at T_a above thermoneutrality (subordinates: $q_{\text{tot}} \approx 2.38$ mW, dominants: $q_{\text{tot}} \approx -0.03$ mW). Given that costs of both thermoregulation and activation of the physiological stress response are expected to be high at our observed sub- and supra-thermoneutral temperatures [e.g. costs of thermoregulation (Grossman and West, 1977; Rising and Hudson, 1974), wherein basal metabolic rate increased by 30% and 40% at our lowest (2.5°C) and highest (38.5°C) observed T_a , respectively; costs of stress response (Depke et al., 2008; Jimeno et al., 2017; reviewed in Sapolsky et al., 2000], such stress-induced changes in T_s and dry heat transfer among subordinate, and thus resource restricted individuals, are perhaps unsurprising.

For stress-exposed subordinates, our observed reduction in q_{tot} by 4.62 mW in the cold, and increase in q_{tot} by 2.38 mW in the heat under stress exposure corresponds with energetic savings of approximately 1.7% and 0.9% of basal metabolic rate, respectively, compared with control subordinates (as estimated for a 10.5 g individual, according to Petit et al., 2013). Although such savings are arguably small, it is likely that stress-induced changes in T_s and q_{tot} extend beyond the eye region alone, therefore rendering our perceived energetic savings underestimates of true, total savings experienced across the whole animal. In most birds, for example, the

majority of environmental heat transfer occurs at exposed integument beyond the eye region (i.e. the legs and bill: Steen and Steen, 1965; Tattersall et al., 2017; Ward et al., 1999); thus, cumulative effects of stress exposure on q_{tot} and subsequent energetic savings are likely to exceed those experienced at the eye region alone, if thermal responses to stress exposure are consistent across exposed integument (as supported by stress-induced declines in wattle and comb temperature of domestic chickens, *Gallus gallus*, held at sub-thermoneutral temperatures, 18°C: Herborn et al., 2015; but see Vianna and Carriave, 2005). Unfortunately, neither the legs nor the bill were readily visible in our thermographic images owing to visual obstruction by flank feathers, and to the relatively large discernible spot-size of our infrared thermographic camera. As such, our ability to explore stress-induced changes in heat transfer and energy allocation at the whole-animal level were limited.

Among our study population, subordinate individuals fed less frequently than dominant individuals across both treatment types (Appendix Fig. A1). Regardless of such differences in resource access between individuals of each social status, thermal consequences of hypophagia alone (i.e. a reduction in the time-averaged heat increment of feeding) appear unlikely to explain differences in stress-induced thermal responses across social hierarchies. On the contrary, T_s of subordinates did not differ from those of dominants at thermoneutral temperatures and even exceeded those of dominants at supra-thermoneutral temperatures – neither of which would be predicted by an elevated heat increment of feeding among dominants. These results suggest that exacerbated thermal responses to stress exposure among subordinates cannot simply be explained by comparative inanition, but rather, seem to reflect a more complex monitoring of energy balance and resource allocation in black-capped chickadees, as hypothesised. Indeed, in our study, subordinates alone suffered declines in mass across stress-exposure treatments (Appendix Fig. A2), suggesting that subordinates but not dominants experienced a negative energy balance at that time (a notable criteria for the emergence of a trade-off; Breuner and Berk, 2019).

Magnitude but not presence of stress-induced thermoregulatory trade-offs depends upon thermal responses at the core

Among our experimental individuals, thermal responses to stress exposure were monitored at surface tissues alone. We, therefore, cannot elucidate whether differences in the magnitude of thermal responses between dominants and subordinates were driven by differences in peripheral vasomotion or differences in core temperature fluctuations. Although neither possibility negates energetic benefits obtained by the responder (here, subordinate individuals), each probably hold differing consequences with respect to individual fitness, and to total energetic savings attributed to stress-induced thermal responses.

At temperatures below thermoneutrality, declines in core body temperature can reduce the metabolic demand of black-capped chickadees by as much as 10% (Reinertsen and Haftorn, 1986), but in doing so, enzymatic activity and neuromuscular response times are also likely to fall (discussed in Brodin et al., 2017) owing to Q_{10} effects. In both mourning doves (*Zenaidura macroura*) and blue tits (*Cyanistes caeruleus*), such declines in neuromuscular response times during hypothermia have been anecdotally and empirically supported, with hypothermic individuals exhibiting reduced flight capacity when compared with normothermic conspecifics (Carr and Lima, 2013; Haftorn, 1972). Consequently, whilst permitting core body temperature to fall under stress exposure and resource

limitation may be an energetically favourable strategy in the cold, negative fitness consequences with respect to predator avoidance may be high (e.g. Metcalfe and Ure, 1995). Contrastingly, although energetic savings acquired by reducing T_s under stress exposure are limited when compared with those obtained by reducing core temperature (1.7% versus 10% of basal metabolic rate, respectively; estimated according to Brodin et al., 2017 and Petit et al., 2013), possible negative fitness consequences of doing so are nearly absent at ecologically relevant temperatures (beyond the risk of regional tissue attrition or reduced functionality).

Similar to stress-induced thermal response in the cold, those observed among subordinates at high T_a (i.e. > the thermoneutral zone; Fig. 3) are also likely to provide energetic advantages irrespective of whether they originate at core tissues, or are isolated to the periphery. At the level of core tissues, for example, permitting hyperthermia at high T_a may allow an individual that is resource limited to both economise on available water and minimise energetic expenses that accompany the act of cooling itself, both evaporatively and behaviourally (Robertson et al., 2020). Such 'adaptive hyperthermia' is a widely used strategy among desert bird species that contend with chronic resource limitations (reviewed in McKechnie and Wolf, 2019), and may well have been employed by social subordinates in our experiment when exposed to rotating stressors. Akin to when hypothermia is employed for resource conservation (discussed above), however, employing hyperthermia at the core for resource conservation is also likely to bear negative fitness consequences in endotherms. In poultry, for example, heat stress has been shown to increase the production of reactive oxygen species (Azad et al., 2010), which in turn may have direct consequences on longevity (Monaghan et al., 2009). In contrast, although energetic savings obtained by increasing T_s at high T_a may be low when compared with those obtained by concurrently elevating core tissue temperatures, long-term negative fitness consequences of doing so are probably negligible.

Importantly, modulation of peripheral vasomotion, and thus dry heat loss at extreme T_a , represents an affordable strategy to reduce thermoregulatory costs. Thus, if T_s responses to stress exposure in our study are driven by peripheral vasomotion alone, the question of why all individuals did not leverage this strategy regardless of experimental treatment or social status is begged. Recent avian studies and classic mammalian studies, however, highlight that constriction or dilation of peripheral vasculature need not be maximised at T_a below or above thermoneutrality, respectively, during waking hours. For example, rapid, vascular-mediated declines in T_s have recently been shown in food-restricted great tits (*Parus major*; Winder et al., 2020) and in stress-exposed Svalbard rock ptarmigans (*Lagopus muta hyperborea*; Nord and Folkow, 2019) despite T_a being below thermoneutrality. Similarly, rapid peripheral vasodilation has been reported in domestic rabbits (*Oryctolagus cuniculus*) despite T_a being above thermoneutrality (Yokoi, 1966). These findings suggest that maintenance of blood flow to surface tissues well within the bounds set by complete vasoconstriction or vasodilation serves additional and critical functions (discussed in Winder et al., 2020) that may be compromised for thermoregulatory benefits during perceived energetic or physical challenges (akin to those contained within our experiment).

Physiology of social dominance alone is unlikely to explain differences in stress-induced thermal responses

A reduction in access to resources among subordinate individuals appears to be the most parsimonious explanation for their

exacerbated T_s responses to stress exposure. However, it is possible that social subordination per se may influence the degree to which physiological processes underpinning the stress-induced thermal response occur. Stress-induced changes in body temperature occur rapidly, and have long been thought to be driven by activation of the sympathetic nervous system (discussed in Jerem et al., 2018). Studies addressing the influence of social status on indirect markers of sympathetic responsiveness (e.g. activation of the hypothalamic–pituitary–adrenal axis; reviewed in Sapolsky et al., 2000), however, provide little support for this explanation. In mountain chickadees (*Poecile gambeli*), for example, subordinate individuals display lower maximal corticosterone release in response to stress exposure than dominant individuals (Pravosudov et al., 2003) – a trend that has also been elucidated in mallards (*Anas platyrhynchos*) and northern pintails (*Anas acuta*) (Poisbleau et al., 2005). Broadly, sympathetic activation is thought to facilitate corticosterone release and activity (reviewed in Sapolsky et al., 2000), suggesting that subordinate individuals should therefore display reduced sympathetic function in addition to reduced corticosterone secretion. Such trends directly contradict our observations of increased stress-induced T_s responses among social subordinates. This suggests that generalised differences in sympathetic physiology correlated with social status are unlikely to explain our results.

Conclusion

In this study, we provide strong suggestive evidence that stress-induced changes in T_s emerge from a trade-off between stress responsiveness and thermoregulation. Specifically, we show that when access to resources is constrained by natural processes (here, social competition), individuals exposed to repeated stressors decrease their dry heat loss at low T_a , and increase their dry heat loss at high T_a (albeit non-significantly) when compared with controls. Such changes in heat transfer are likely to provide benefits by reducing demand on thermogenesis in the cold, or evaporative cooling in the heat. In stark contrast, when resources are not constrained (as observed in dominant individuals), exposure to repeated stressors elicits negligible effects on patterns of T_s and dry-heat loss across T_a . An understanding of the precise degree to which thermoregulatory expenses are offset under stress exposure and resource constraint, however, remains unclear, and will require a more holistic knowledge of regional body temperature changes. Together, our results provide further support for a functional value of stress-induced changes in T_s that, to date, has been largely unexplored. More importantly, our findings raise concerns about the capacity of endotherms to cope with the combined challenges of rising climatic instability and frequency of perceived challenges that emerge from an increasingly human-populated and urbanised world.

Appendix

Enumeration of video observations and categorisation of social interactions

To test whether thermal responses to stress exposure differed between dominant and subordinate black-capped chickadees, we required estimation of linear dominance hierarchies within each experimental flight enclosure and, subsequently, individual social positions within each hierarchy. Typically, studies estimating dominance hierarchies from video observations rely upon subjective categorisations of behaviours as agonistic or non-agonistic (Silva et al., 2018; Snijders et al., 2016) and, consequently, are likely to suffer from observer bias. To eliminate

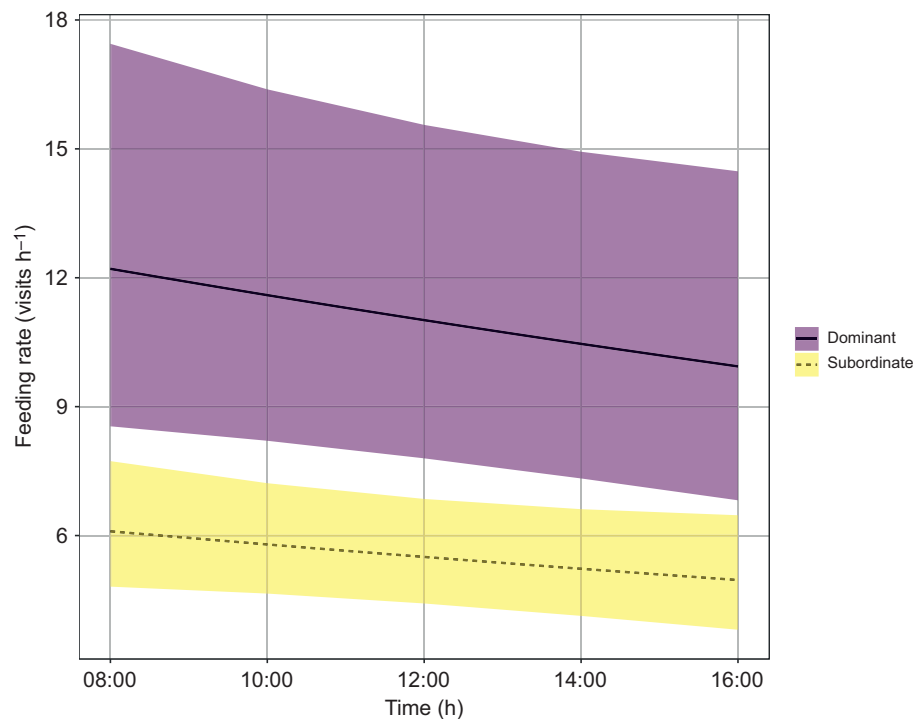


Fig. A1. Effects of social status and stress exposure on feeding rate of black-capped chickadees across time of day. Feeding rate (marginal mean from generalised additive mixed-effects model) was calculated from video observations made across 60 days, with a minimum of 1 h of observation per flight enclosure ($n=4$ flight enclosures), where individuals were identified by coloured leg bands. Solid lines represent mean feeding rate of socially dominant individuals and dashed lines represent that of socially subordinate individuals. Purple and yellow bands represent 95% confidence intervals (CIs) around feeding rate estimates for socially dominant and socially subordinate individuals, respectively. Feeding rate significantly differed between treatments ($P=0.007$), and across social hierarchies ($P=0.001$), at $\alpha=0.05$.

observer bias from our estimates of dominance hierarchies, we chose to enumerate video observations as presence and absence data across time (per individual), then apply static and consistent criteria to infer and categorise social interactions without requiring visual observation of their occurrence (see Materials and Methods, 'Social status determination'). Here, video observations were converted to enumerated time-line data by determining the presence of individuals at feeding platforms across video frames, then assigning each presence and absence per individual a binomial value (present=1, absent=0) for a given second of observation.

The presence of individuals at feeding platforms was determined by detecting arrivals and departures from video recordings using automated motion detection, then identifying individuals present

using coloured leg-band combinations. Specifically, we scanned videos for the presence of motion (defined as a change in pixel content between adjacent frames exceeding 1.75%, at a frame rate of 8 frames s⁻¹) in R (<http://www.R-project.org/>), then extracted still frames from 2 s around the time of motion (8 frames s⁻¹; $n=16$ frames). Individuals observed to be arriving to, present at and departing from feeding platforms within extracted frames were then identified by observation of coloured leg-band combinations, and arrival, presence and departure times were documented according to real time (as determined from digital time stamps of parallel thermographic images).

In our study, we defined agonistic interactions as displacement and waiting events from feeding platforms (similar to Evans et al.,

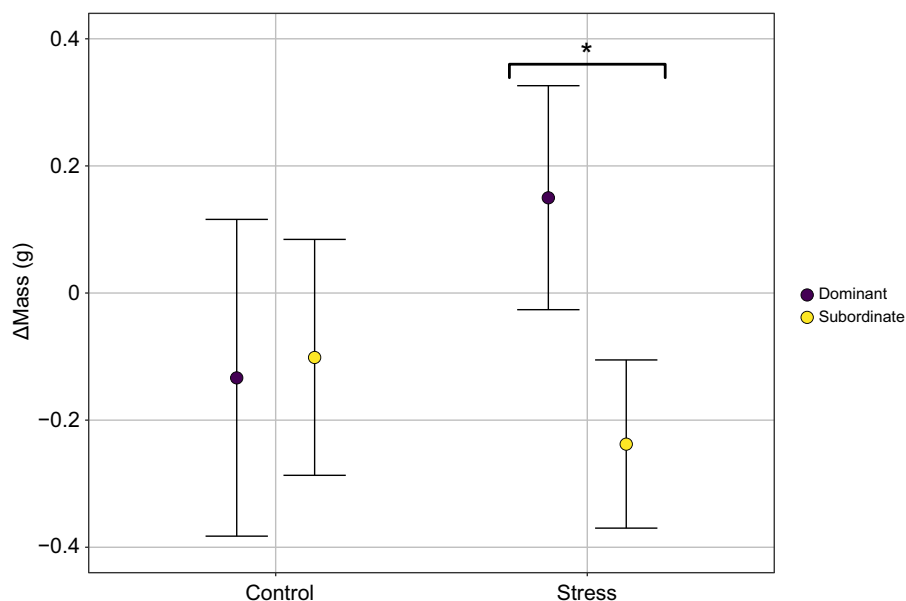


Fig. A2. Effect of experimental treatment type on change in mass (across 30 days) of socially dominant and socially subordinate individuals. Mass of each individual ($n=6$ dominant, $n=11$ subordinate) was measured at the onset and completion of each treatment. Purple dots and yellow dots represent mean change in mass (Δmass , marginal means from linear mixed-effects model) of socially dominant and socially subordinate individuals, respectively. Socially subordinate individuals lost significantly more mass than socially dominant individuals across stress-exposure treatments according to planned comparison ($*P=0.001$; $\alpha=0.05$). Whiskers represent 95% CIs around means.

2018), whilst non-agonistic interactions included the prolonged and simultaneous presence of multiple individuals at a single feeding platform. We defined displacement events as social interactions wherein the arrival of one individual to a feeding platform (individual A) stimulated the departure of another individual that was previously present at the same feeding platform (individual B), within 1 s of individual A's arrival. Recently, Evans et al. (2018) have shown that social ranks estimated from raw video and enumerated presence/absence data were most similar when the criteria for displacement events were limited such that the minimum length of time that individual A must remain at the feeding platform following its arrival (j) was 5 s. Identification of displacement events in our study was therefore limited such that $j \geq 5$ s. We defined waiting events as social interactions wherein the arrival of individual A to a feeding platform was contingent upon the departure of individual B from the same feeding platform, and occurred between 1 and 3 s after the departure of individual B. Unlike our criteria for displacement events, no minimum retention time of individual A was defined (i.e. $j > 0$ s). Non-agonistic encounters were defined as the simultaneous presence of two individuals at a feeding platform for more than 2 s.

Effect of social status on feeding rate and stress-induced mass loss

Previous studies have reported a positive correlation between social dominance and priority of access to supplemental food among black-capped chickadees (e.g. Ficken et al., 1990; Smith, 1991); others have reported a positive correlation between social dominance and mass (van Oort et al., 2007; but see Schubert et al., 2007). In this study, our predictions were contingent upon such correlations being detectable within our experimental population. We therefore tested whether social status influenced both the feeding rate and change in mass of individuals across the duration of a given treatment (i.e. stress exposure or control).

To test whether social status influenced feeding rate among individuals, we used a GAMM in the R package 'mgcv' (Wood, 2011) with feeding rate (number of visits per hour of digital video observation) as the response variable and social status, treatment type (to account for an influence of stress exposure on feeding behaviour in birds Favreau-Peigné et al., 2014) and sex as parametric predictors. In this model, both T_a (mean per hour) and time of day (hour) were also included as predictors with head being initially modelled non-linearly (cubic regression splines; knots=4) to capture expected non-linear trends in feeding rate across each (Bonter et al., 2013; Brittingham and Temple, 1992). Our results, however, provided little evidence for a curvilinear relationship between time of day and feeding rate within our observations. Time of day was therefore subsequently included as a linear predictor. Finally, interactions between both treatment and time of day, and treatment and mean T_a were also included to account for possible time- and temperature-dependent feeding patterns following stress exposure, and an interaction between social status and treatment was included to test for possible differences in behavioural responses to stress exposure across hierarchies. Date (Julian), an individual's capture locale (1 of 6) and bird identity were included as random intercepts to account for variance explained by each alone. In our data, variance in feeding rate exceeded mean feeding rate ($\lambda > \mu$). We therefore assumed a negative binomial distribution to capture over-dispersion ($\phi = 2.4$).

The results of our GAMM showed that socially subordinate individuals fed less than socially dominant individuals ($\beta \pm \text{s.e.m.} = -0.811 \pm 0.247$, $t = -3.287$, $P = 0.001$; Fig. A1), regardless

of treatment type (status by treatment interaction: $\beta = 0.113 \pm 0.096$, $t = 1.181$, $P = 0.238$; Fig. A1). Feeding rate significantly declined across time of day under control conditions ($\beta = -0.057 \pm 0.021$, $t = -2.712$, $P = 0.007$; Fig. A1), but not stress-exposure conditions (treatment:time of day: $\beta = 0.063 \pm 0.028$, $t = 2.192$, $P = 0.029$; Fig. A1), and those experiencing stress-exposure treatment fed less than controls regardless of time of day ($\beta = 3.328 \pm 0.445$, $t = 7.483$, $P < 0.001$; Fig. A1). Feeding rate significantly varied across T_a ($f = 2.192$, estimated degrees of freedom, e.d.f. = 2.786, $P = 0.022$), with individuals displaying a bimodal response across our observed temperature range. Again, this trend was significantly altered by the presence of stress exposure ($f = 4.540$, e.d.f. = 1.009, $P = 0.032$), such that the amplitude of each mode was reduced under stress-exposure conditions when compared with control conditions. An effect of sex on feeding rate was not significant ($\beta = -0.2140.256$, $t = -0.836$, $P = 0.403$).

To test whether social status influenced an individual's change in mass across a given treatment, we used a linear mixed-effects model (LMM) in the R package 'glmmTMB' (Brooks et al., 2017), with change in mass between the termination and onset of each treatment (Δmass) as the Gaussian distributed response variable ($n_s = 17$ individuals, $n = 6$ socially dominant, $n = 11$ socially subordinate). In this model, both social status and treatment were included as fixed predictors, and an interaction between social status and treatment was included to account for possible differences in the effect of social status on mass between treatment types. Sex was included as an additional fixed predictor to control for differences in food acquisition rate that may exist between sexes, regardless of social status. Both individual identity and flight enclosure identity were initially included as random intercepts to account for variance explained by each parameter; however, flight enclosure identity was later removed because it explained negligible variance in our data (s.d. < 0.001). Finally, because previous research has suggested that the effect of stress exposure on feeding behaviour in birds can vary substantially among individuals (Favreau-Peigné et al., 2014), we allowed the variance in our model to differ according to treatment type by weighting according to binomial treatment.

Mass did not significantly change across control treatments ($n = 30$ days) for both socially subordinate ($\beta = 0.032 \pm 0.151$, $z = 0.212$, $P = 0.832$) and socially dominant individuals ($\beta = -0.129 \pm 0.129$, $z = -1.001$, $P = 0.317$). Under stress-exposure treatment, however, the mass of socially subordinate individuals significantly fell (treatment:social status $\beta = -0.420 \pm 0.185$, $z = -2.270$, $P = 0.023$; Fig. A2), whilst that of socially dominant individuals increased ($\beta = 0.283 \pm 0.149$, $z = 1.905$, $P = 0.057$; Fig. A2), albeit non-significantly. Final change in mass across stress-exposure treatments alone significantly differed between socially subordinate and socially dominant individuals, according to a planned comparison ($\beta = 0.388 \pm 0.107$, $t_{27} = -3.609$, $P = 0.001$). Sex did not influence change in mass across treatments ($\beta = -0.009 \pm 0.085$, $z = -0.105$, $P = 0.916$). Mean ($\pm \text{s.e.m.}$) change in mass among social subordinates was -0.238 ± 0.065 g, and among social dominants was 0.150 ± 0.086 g.

Acknowledgements

We thank Lianne Ralph for assistance in capturing black-capped chickadees and conducting experimental manipulations, Glenn Tattersall for thermographic advice, Simon Tapper and Robby Marotte for statistical advice, James Quinn for theoretical discussions and generous contribution of laboratory space, and Simon Tapper, Kimberley Tasker and Tyler Maksymiw for their assistance in aviary construction. We sincerely thank the staff and avian banding team at the Ruthven Park National Historic Site for providing a space to construct our aviary, and providing hospitality during experimentation.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.K.R.; Methodology: J.K.R.; Formal analysis: J.K.R.; Data curation: J.K.R.; Writing - original draft: J.K.R.; Writing - review & editing: G.F.M., G.B.; Visualization: J.K.R.; Supervision: G.F.M., G.B.; Funding acquisition: G.F.M., G.B.

Funding

All funding for this research was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) to G.B. (RGPIN-04158-2014), and by a Natural Sciences and Engineering Research Council of Canada Collaborative Research and Training Experience Program (CREATE 481954-2016).

Data availability

All data and R code used for the construction of this study are available from the Dryad digital repository (Robertson, 2020): dryad.rjf6q5774

References

- Aghajani, M., Vaez Mahdavi, M., Khalili Najafabadi, M., Ghazanfari, T., Azimi, A., Mahdi Dust, S. and Arbab Soleymaniand, S. (2013). Effects of dominant/subordinate social status on formalin-induced pain and changes in serum proinflammatory cytokine concentrations in mice. *PLoS ONE* **8**, e80650. doi:10.1371/journal.pone.0080650
- Andreasson, F., Nord, A. and Nilsson, J. (2019). Body temperature responses of great tits *Parus major* to handling in the cold. *Ibis* **162**, 836-844. doi:10.1111/ibi.12737
- Azad, A., Kikusato, M., Hoque, A. and Toyomizu, M. (2010). Effect of chronic heat stress on performance and oxidative damage in different strains of chickens. *J. Poult. Sci.* **47**, 333-337. doi:10.2141/jpsa.010025
- Bartlett, W. (1912). An experimental study of the arteries in shock. *J. Exp. Med.* **15**, 415-428. doi:10.1084/jem.15.4.415
- Best, R. and Fowler, R. (1981). Infrared emissivity and radiant surface temperatures of Canada and snow geese. *J. Wildl. Manag.* **45**, 1026-1029. doi:10.2307/3808122
- Bonter, D., Zuckerberg, B., Sedgwick, C. and Hochachka, W. (2013). Daily foraging patterns in free-living birds: Exploring the predation-starvation trade-off. *Proc. R. Soc. B* **280**, 20123087. doi:10.1098/rspb.2012.3087
- Breuner, C. and Berk, S. (2019). Using the van Noordwijk and de Jong resource framework to evaluate glucocorticoid-fitness hypotheses. *Int. Comp. Biol.* **59**, 243-250. doi:10.1093/icb/icz088
- Brittingham, M. and Temple, S. (1992). Use of winter bird feeders by black-capped chickadees. *J. Wildl. Manag.* **55**, 103-111. doi:10.2307/3808797
- Brodin, A., Nilsson, J. and Nord, A. (2017). Adaptive temperature regulation in the little bird in winter: Predictions from a stochastic dynamic programming model. *Oecologia* **185**, 43-54. doi:10.1007/s00442-017-3923-3
- Brooks, M., Kristensen, K., van Benthem, K., Magnusson, A., Berg, C., Skaug, A. N. H., Mächler, M. and Bolker, B. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378-400. doi:10.32614/RJ-2017-066
- Burness, G., Armstrong, C., Fee, T. and Tilman-Schnidell, E. (2010). Is there an energetic-based trade-off between thermoregulation and the acute phase response in zebra finches? *J. Exp. Biol.* **213**, 1386-1394. doi:10.1242/jeb.027011
- Cabanac, M. and Gosselin, F. (1993). Emotional fever in the lizard *Callopiastes maculatus* (teiididae). *Anim. Behav.* **46**, 200-200. doi:10.1006/anbe.1993.1178
- Calisi, R., Rizzo, N. and Bentley, G. (2008). Seasonal differences in hypothalamic egr-1 and gnih expression following capture-handling stress in house sparrows (*Passer domesticus*). *Gen. Comp. Endocrinol.* **157**, 283-287. doi:10.1016/j.ygcen.2008.05.010
- Cannon, W. (1932). *The Wisdom of the Body*. New York, USA: Norton; Company Inc.
- Carr, J. and Lima, S. (2013). Nocturnal hypothermia impairs flight ability in birds: A cost of being cool. *Proc. R. Soc. B Biol. Sci.* **280**, 20131846. doi:10.1098/rspb.2013.1846
- Depke, M., Fusch, G., Domanska, G., Geffers, R., Völker, U., Schuett, C. and Kiank, C. (2008). Hypermetabolic syndrome as a consequence of repeated psychological stress in mice. *Endocrinol.* **149**, 2714-2723. doi:10.1210/en.2008-0038
- Desrochers, A., Hannon, S. J. and Nordin, K. E. (1988). Winter survival and territory acquisition in a northern population of Black-capped Chickadees. *Auk* **105**, 727-736. doi:10.1093/auk/105.4.727
- Dymond, K. and Fewell, J. (1998). Gender influences the core temperature response to a simulated open field in adult guinea pigs. *Physiol. Behav.* **65**, 889-892. doi:10.1016/S0031-9384(98)00198-X
- Elo, A. (1978). *The Rating of Chessplayers, Past and Present*. New York, USA: Arco Pub.
- Evans, J., Devost, I., Jones, T. and Morand-Ferron, J. (2018). Inferring dominance interactions from automatically recorded temporal data. *Ethol* **124**, 188-195. doi:10.1111/eth.12720
- Favreau-Peigné, A., Calandreau, L., Constantin, P., Gaultier, B., Bertin, A., Arnould, C., Laurence, A., Richard-Yris, M., Houdelier, C., S. S. (2014). Emotionality modulates the effect of chronic stress on feeding behaviour in birds. *PLoS ONE* **9**, e87249. doi:10.1371/journal.pone.0087249
- Ficken, M., Weise, C. and Popp, J. (1990). Dominance rank and resource access in winter flocks of black-capped chickadees. *Wilson Bull.* **102**, 623-633.
- Fox, J. and Weisberg, S. (2019). *An R Companion to Applied Regression*, 3rd edn. Thousand Oaks, USA: Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- Fridolfsson, A. and Ellegren, H. (1999). A simple and universal method for molecular sexing of non-ratite birds. *J. Avian Biol.* **30**, 116-121. doi:10.2307/3677252
- Glase, J. (1973). Ecology of social organization in the black-capped chickadee. *Living Bird* **12**, 235-267.
- Gould, S. J. and Lewontin, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. B* **205**, 581-598. doi:10.1098/rspb.1979.0086
- Grachev, P., Li, X. and O'Byrne, K. (2013). Stress regulation of kisspeptin in the modulation of reproductive function. In *Kisspeptin Signaling in Reproductive Biology* (ed. A. Kauffman and J. Smith), pp. 431-454. New York, USA: Springer.
- Greenacre, C. and Lusby, A. (2004). Physiologic responses of amazon parrots (*Amazona species*) to manual restraint. *J. Avian Med. Surg.* **18**, 19-23. doi:10.1647/2003-011
- Griffiths, R., Dean, S. and Dijkstra, C. (1996). Sex identification in birds using two chd genes. *Proc. R. Soc. Lond. B* **263**, 1251-1256. doi:10.1098/rspb.1996.0184
- Grossman, A. and West, G. (1977). Metabolic rate and temperature regulation of winter acclimatized black-capped chickadees *Parus atricapillus* of interior Alaska. *Ornis Scand* **8**, 127-138. doi:10.2307/3676097
- Haftorn, S. (1972). Hypothermia of tits in the arctic winter. *Ornis Scand* **3**, 153-166. doi:10.2307/3676222
- Herborn, K., Graves, J., Jerem, P., Evans, N., Nager, R., McCafferty, D. and McKeegan, D. (2015). Skin temperature reveals the intensity of acute stress. *Physiol. Behav.* **152**, 225-230. doi:10.1016/j.physbeh.2015.09.032
- Jerem, P., Herborn, K., McCafferty, D., McKeegan, D. and Nager, R. (2015). Thermal imaging to study stress non-invasively in unrestrained birds. *J. Vis. Exp.* **6**, e53184. doi:10.3791/53184
- Jerem, P., Jenni-Eiermann, S., Herborn, K., McKeegan, D., McCafferty, D. and Nager, R. (2018). Eye region surface temperature reflects both energy reserves and circulating glucocorticoids in a wild bird. *Sci. Rep.* **8**, 1907. doi:10.1038/s41598-018-20240-4
- Jerem, P., Jenni-Eiermann, S., McKeegan, D., McCafferty, D. and Nager, R. (2019). Eye region surface temperature dynamics during acute stress relate to baseline glucocorticoids independently of environmental conditions. *Physiol. Behav.* **210**, 112627. doi:10.1016/j.physbeh.2019.112627
- Jimeno, B., Hau, M. and Verhulst, S. (2017). Strong association between corticosterone levels and temperature-dependent metabolic rate in individual zebra finches. *J. Exp. Biol.* **220**, 4426-4431. doi:10.1242/jeb.166124
- Kataoka, N., Hioki, H., Kaneko, T. and Nakamura, K. (2014). Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. *Cell Metab.* **20**, 346-358. doi:10.1016/j.cmet.2014.05.018
- King, M. and Swanson, D. (2013). Activation of the immune system incurs energetic costs but has no effect on the thermogenic performance of house sparrows during acute cold challenge. *J. Exp. Biol.* **216**, 2097-2102. doi:10.1242/jeb.079574
- Kinsey-Jones, J. S., Li, X. F., Knox, A. M. I., Wilkinson, E. S., Zhu, X. L., Chaudhary, A. A., Milligan, S. R., Lightman, S. L. and O'Byrne, K. T. (2009). Down-regulation of hypothalamic kisspeptin and its receptor, kiss1r, mRNA expression is associated with stress-induced suppression of luteinising hormone secretion in the female rat. *J. Neuroendocrinol.* **21**, 20-29. doi:10.1111/j.1365-2826.2008.01807.x
- Kirby, E. D., Geraghty, A. C., Ubuka, T., Bentley, G. E. and Kaufer, D. (2008). Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proc. Natl. Acad. Sci. USA* **106**, 11324-11329. doi:10.1073/pnas.0901176106
- Knauber, J., El-Madany, T., Zaehle, S. and Migliavacca, M. (2018). Bogleaf - an R package for the calculation of physical and physiological ecosystem properties from eddy covariance data. *PLoS ONE* **13**, e0201114. doi:10.1371/journal.pone.0201114
- Lewden, A., Nord, A., Petit, M. and Vézina, F. (2017). Body temperature responses to handling stress in wintering black-capped chickadees (*Poecile atricapillus*). *Physiol. Behav.* **179**, 49-54. doi:10.1016/j.physbeh.2017.05.024
- Martin, L., Scheuerlein, A. and Wikelski, M. (2003). Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proc. R. Soc. B* **270**, 153-158. doi:10.1098/rspb.2002.2185
- McCafferty, D., Gilbert, C., Paterson, W., Pomeroy, P., Thompson, D., Currie, J. and Ancel, A. (2011). Estimating metabolic heat-loss in birds and mammals by combining infrared thermography with biophysical modelling. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **158**, 337-345. doi:10.1016/j.cbpa.2010.09.012
- McEwen, B. (1998). Stress, adaptation, and disease: allostasis and allostatic load. *Am. NY Acad. Sci.* **840**, 33-44. doi:10.1111/j.1749-6632.1998.tb09546.x

- McKechnie, A. and Wolf, B.** (2019). The physiology of heat tolerance in small endotherms. *Physiol* **34**, 302–313. doi:10.1152/physiol.00011.2019
- McKechnie, A., Gerson, A., McWhorter, T., Smith, E., Talbot, W. and Wolf, B.** (2016). Avian thermoregulation in the heat: Evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *J. Exp. Biol.* **220**, 2436–2444. doi:10.1242/jeb.155507
- Merlo, J., Cutrera, A., Luna, F. and Zenuto, R.** (2014). PHA-induced inflammation is not energetically costly in the subterranean rodent *Ctenomys talarum* (tucutucos). *Comp. Biochem. Physiol. A Mol. Int. Physiol.* **175**, 90–95. doi:10.1016/j.cbpa.2014.05.021
- Metcalfe, N. B. and Ure, S. E.** (1995). Diurnal variation in flight performance and hence potential predation risk in small birds. *Proc. R. Soc. B* **261**, 395–400. doi:10.1098/rspb.1995.0165
- Minkina, W. and Dudzik, S.** (2009). *Infrared Thermography Errors and Uncertainties*. Chichester, UK: Wiley Press.
- Monaghan, P., Metcalfe, N. and Torres, R.** (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75–92. doi:10.1111/j.1461-0248.2008.01258.x
- Nord, A. and Folkow, L.** (2019). Ambient temperature effects on stress-induced hyperthermia in svalbard ptarmigan. *Biol. Open* **8**, bio043497. doi:10.1242/bio.043497
- Nord, A. and Nilsson, J.-A.** (2019). Heat dissipation rate constrains reproductive investment in a wild bird. *Funct. Ecol.* **33**, 250–259. doi:10.1111/1365-2435.13243
- Nord, A., Sandell, M. and Nilsson, J.-A.** (2010). Female zebra finches compromise clutch temperature in energetically demanding incubation conditions. *Funct. Ecol.* **24**, 1031–1036. doi:10.1111/j.1365-2435.2010.01719.x
- Oka, T., Oka, K. and Hori, T.** (2001). Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom. Med.* **63**, 476–486. doi:10.1097/00006842-200105000-00018
- Ots, I., Kerimov, A., Ivankina, E., Ilyina, T. and Hórak, P.** (2001). Immune challenge affects basal metabolic activity in wintering great tits. *Proc R Soc B Biol. Sci.* **268**, 1175–1181. doi:10.1098/rspb.2001.1636
- Parr, L. A. and Hopkins, W. D.** (2000). Brain temperature asymmetries and emotional perception in chimpanzees, *Pantroglydotes*. *Physiol. Behav.* **71**, 363–371. doi:10.1016/S0031-9384(00)00349-8
- Petit, M., Lewden, A. and Vézina, F.** (2013). Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and thermogenic capacity. *PLoS ONE* **8**, e68292. doi:10.1371/journal.pone.0068292
- Poisbleau, M., Fritz, H., Guillon, N. and Chastel, O.** (2005). Linear social dominance hierarchy and corticosterone responses in male mallards and pintails. *Horm. Behav.* **47**, 485–492. doi:10.1016/j.yhbeh.2005.01.001
- Pravosudov, V., Mendoza, S. and Clayton, N.** (2003). The relationship between dominance, corticosterone, memory, and food caching in mountain chickadees (*Parus gambeli*). *Horm. Behav.* **44**, 93–102. doi:10.1016/S0018-506X(03)00119-3
- Proctor, C., Freeman, E. and Brown, J.** (2010). Influence of dominance status on adrenal activity and ovarian cyclicity status in captive African elephants. *Zoo Biol.* **29**, 168–178. doi:10.1002/zoo.20292
- Reinertsen, R. and Haftorn, S.** (1984). The effect of short-time fasting on metabolism and nocturnal hypothermia in the willow tit, *Parus montanus*. *J. Comp. Physiol. B* **154**, 23–28. doi:10.1007/BF00683212
- Reinertsen, R. and Haftorn, S.** (1986). Different metabolic strategies of northern birds for nocturnal survival. *J. Comp. Physiol. B* **156**, 655–663. doi:10.1007/BF00692743
- Rey, S., Huntingford, F., Boltana, S., Vargas, R., Knowles, T. and Mackenzie, S.** (2015). Fish can show emotional fever: Stress-induced hyperthermia in zebrafish. *Proc. R. Soc. B* **282**, 20152266. doi:10.1098/rspb.2015.2266
- Rich, E. and Romero, L.** (2005). Exposure to chronic stress downregulates corticosterone responses to acute stressors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R1628–R1636. doi:10.1152/ajpregu.00484.2004
- Rising, J. and Hudson, J.** (1974). Seasonal variation in the metabolism and thyroid activity of the black-capped chickadee (*parus atricapillus*). *Condor* **76**, 198–203. doi:10.2307/1366730
- Robertson, J.** (2020). Social hierarchy reveals thermoregulatory trade-offs in response to repeated stressors. Dryad, Dataset. doi:10.5061/dryad.rf6q5774
- Robertson, J., Mastromonaco, G. and Burness, G.** (2020). Evidence that stress-induced changes in surface temperature serve a thermoregulatory function. *J. Exp. Biol.* **223**, jeb213421. doi:10.1242/jeb.213421
- Romero, L., Dickens, M. and Cyr, N.** (2009). The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* **55**, 375–389. doi:10.1016/j.yhbeh.2008.12.009
- Sánchez-Tójar, A., Schroeder, J. and Farine, D.** (2018). A practical guide for inferring reliable dominance hierarchies and estimating their uncertainty. *J. Anim. Ecol.* **87**, 594–608. doi:10.1111/1365-2656.12776
- Sapolsky, R.** (2004). *Why Zebras don't get Ulcers: The Acclaimed Guide to Stress, Stress-Related Diseases, and Coping—Now Revised and Updated*. New York, USA: Holt Publishing.
- Sapolsky, R., Romero, L. and Munck, A.** (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrin. Rev.* **21**, 55–89. doi:10.1210/er.21.1.55
- Schubert, K., Mennil, D., Ramsay, S., Otter, K., Boag, P. and Ratcliffe, L.** (2007). Variation in social rank acquisition influences lifetime reproductive success in black-capped chickadees. *Biol. J. Linnean. Soc.* **90**, 85–95. doi:10.1111/j.1095-8312.2007.00713.x
- Selye, H.** (1950). Stress and the general adaptation syndrome. *BMJ* **1**, 1383. doi:10.1136/bmj.1.4667.1383
- Seutin, G., White, B. and Boag, P.** (1991). Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* **69**, 82–90. doi:10.1139/z91-013
- Silva, L., Lardy, S., Ferreira, A., Rey, B., Doutrelant, C. and Covas, R.** (2018). Females pay the oxidative cost of dominance in a highly social bird. *Anim. Behav.* **114**, 135–146. doi:10.1016/j.anbehav.2018.08.006
- Simpson, G.** (2018). Modelling palaeoecological time series using generalised additive models. *Front. Ecol. Evol.* **6**, 149. doi:10.3389/fevo.2018.00149
- Smith, S.** (1991). *The black-capped chickadee. Behavioral ecology and Natural History*. Ithica, USA: Cornell University Press.
- Snijders, L., Naguib, M. and van Oers, K.** (2016). Dominance rank and boldness predict social attraction in great tits. *Behav. Ecol.* **28**, 398–406. doi:10.1093/beheco/arw158
- Speakman, J.** (2008). The physiological costs of reproduction in small mammals. *Phil. Trans. R. Soc. B* **363**, 375–398. doi:10.1098/rstb.2007.2145
- Stearns, S. C.** (1992). *The Evolution of Life Histories*. London, UK: Oxford University Press.
- Steen, I. and Steen, J.** (1965). The importance of the legs in the thermoregulation of birds. *Acta Physiol. Scand.* **63**, 285–291. doi:10.1111/j.1748-1716.1965.tb04067.x
- Stier, K., Almasi, B., Gasparini, J., Piault, R., Roulin, A. and Jenni, L.** (2009). Effects of corticosterone on innate and humoral immune functions and oxidative stress in barn owl nestlings. *J. Exp. Biol.* **212**, 2085–2091. doi:10.1242/jeb.024406
- Svensson, E., Råberg, L., Koch, C. and Hasselquist, D.** (1998). Energetic stress, immunosuppression and the costs of an antibody response. *Funct. Ecol.* **12**, 912–919. doi:10.1046/j.1365-2435.1998.00271.x
- Tattersall, G.** (2016). Infrared thermography: a non-invasive window into thermal physiology. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **202**, 78–98. doi:10.1016/j.cbpa.2016.02.022
- Tattersall, G., Arnaout, B. and Symonds, M.** (2017). The evolution of the avian bill as a thermoregulatory organ. *Biol. Rev.* **92**, 1630–1656. doi:10.1111/brv.12299
- Van Hooff, J. A. R. A. M. and Wensing, J. A. B.** (1987). Dominance and its behavioural measures in a captive wolf pack. In *Man and Wolf* (ed. H. W. Frank), pp. 219–252. Dordrecht: Junk Publishers.
- van Oort, H., Otter, K., Fort, K. and McDonnell, Z.** (2007). Habitat, dominance, and the phenotypic quality of male black-capped chickadees. *Condor* **109**, 88–96. doi:10.1093/condor/109.1.88
- Vianna, D. and Carrive, P.** (2005). Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. *Euro. J. Neurosci.* **21**, 2505–2512. doi:10.1111/j.1460-9568.2005.04073.x
- Vleck, C.** (1981). Energetic cost of incubation in the zebra finch. *Condor* **83**, 229–237. doi:10.2307/1367313
- Ward, S., Rayner, J., Möller, U., Jackson, D., Nachtigall, W. and Speakman, J.** (1999). Heat transfer from starlings *Sturnus vulgaris* during flight. *J. Exp. Biol.* **202**, 1589–1602.
- Winder, L. A., White, S. A., Nord, A., Helm, B. and McCafferty, D. J.** (2020). Body surface temperature responses to food restriction in wild and captive great tits. *J. Exp. Biol.* **223**, jeb220046. doi:10.1242/jeb.220046
- Wood, S.** (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat Soc. B* **73**, 3–36. doi:10.1111/j.1467-9868.2010.00749.x
- Yeo, I.** (2005). Hippocrates in the context of Galen: Galen's commentary on the classification of fevers in epidemics vi. *Stud. Anc. Med.* **31**, 433–443. doi:10.1163/9789004377271_027
- Yokoi, Y.** (1966). Effect of ambient temperature upon emotional hyperthermia and hypothermia in rabbits. *J. Appl. Physiol.* **21**, 1795–1798. doi:10.1152/jappl.1966.21.6.1795