Thermoregulatory consequences of growing up during a heatwave

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Abstract

Climate change is causing an increase in the frequency and intensity of heatwaves. In birds, changes in environmental temperature during the developmental period can affect growth, metabolism, and temperature tolerance of the offspring. We know little about whether such changes remain to adulthood, which is important to understand the links between climate change, development, and fitness. We investigated if phenotypic consequences of the thermal environment in early life remained in adulthood by raising Japanese quail (*Coturnix japonica*) under simulated heatwave- or control conditions from hatching until halfway through the growth period, and then in a common garden until reproductively mature. We measured biometric and thermoregulatory (metabolic heat production [MHP], evaporative water and heat loss [EWL, EHL] and body temperature) responses to variation in ambient temperature at the end of the heatwave treatment and in adulthood. Developmental temperature did not affect body mass or wing length. However, warm birds had lower MHP than control birds at the end of the heat wave period and, in the warmest temperature studied (40°C), warm birds had higher evaporative cooling capacity than control birds. None of the effects found at the end of the heatwave period remained until adulthood. This implies that chicks exposed to higher temperatures could be more prepared to counter heat stress as juveniles, but that they do not enjoy any advantages of such developmental conditions when facing high temperatures as adults.

Introduction

Climate change, including increased global mean temperature and increased climatic instability, has strongly impacted animal populations, for example, through changes in species distributions' (Chen et al., 2011), mass mortality events (McKechnie et al., 2012) and population extinctions (Thomas et al., 2006). A warmer climate is also associated with a range of sublethal consequences that can negatively affect fitness (Conradie et al., 2019). For example, many insectivorous passerines in the Northern Hemisphere have advanced breeding start to match a warming-related advancement of peak food abundance, but still pay fitness costs since phenotypic plasticity in egg laying cannot fully compensate for the earlier emergence of insect prey (Shipley et al., 2020; Both et al., 2009; Visser et al., 1998; Hansson et al., 2014). Moreover, when warming is acute and pronounced, such as during a heatwave, parents might have to trade-off workload against the risk of overheating (Cunningham et al., 2013; du Plessis et al., 2012; Nilsson and Nord, 2018) with implications for both current and future reproduction (Sharpe et al., 2019; Cunningham et al., 2021; Nord & Nilsson, 2019; Andreasson et al., 2020). Understanding the ontogeny and evolution of animals' ability to cope with fluctuating temperatures is thus fundamental to predict the consequences of climate change on wildlife (McKechnie and Wolf, 2019; Albright et al., 2017).

Changes in temperature during the developmental period before or after hatching can affect subsequent temperature tolerance (reviewed by Nord and Giroud, 2020). Conceptually, it can be hypothesised that programming effects of developmental temperature on adult thermoregulation fall on a scale between constraining and priming: 1) if early-life temperature programs phenotypes that perform optimally in that thermal environment, adults should maximise fitness when early- and later-life environments are similar ("environmental matching hypothesis"; reviewed by Monaghan, 2008); 2) if deviations from optimal developmental conditions act suppressively on the phenotype, then increasing distance from optimality in early life should come at a fitness cost regardless of the adult thermal environment ("silver spoon hypothesis"; Monaghan, 2008). In line with the environmental matching hypothesis, rats reared in warmer conditions grew their tails longer and feet larger and reduced the amount of body fat, which was interpreted as adaptions to facilitate heat loss (Demicka and Caputa, 1993). However, in wild systems, exposure to warmer developmental temperatures have been shown to have negative effects on nestling growth and fledging success; heat-stressed chicks weighed less and grew more slowly than individuals reared in control conditions (Deaton et al., 1978; Andreasson et al., 2018; Rodriguez and Barba, 2016; Cunningham et al., 2013; van de Ven et al., 2020; Andrew et al., 2017). These findings conform more closely with the silver spoon hypothesis. However, other studies demonstrate positive effects of warmer developmental temperatures on nestling growth and fledgling success (McCarty and Winkler, 1999; Dawson et al., 2005). For example, tree swallow (*Tachycineta bicolor*) nestlings gained more mass due to both higher temperatures and higher food abundance (which, in turn, was also

positively affected by higher temperatures) (McCarty and Winkler, 1999), and both nestling survival and fledging success increased with higher temperatures in another study on the same species (Dawson et al., 2005).

We know less about the physiological basis of any matching between developmental and adult thermal environments (reviewed by Nord & Giroud, 2020). Studies on poultry suggest that precisely timed and dosed thermal stimuli during embryonic or perinatal development can lead to epigenetic programming that makes the birds better at withstanding matched temperature stressors, such as altered body temperature (T_b) and metabolic rate, that last at least until adolescence (Nichelmann, 2004; reviewed by e.g. Farag and Alagawany, 2018). There are few comparable studies in wild systems. However, Andreasson et al. (2018) found that blue tit (*Cyanistes caeruleus*) nestlings from heated nest boxes had higher T_b and grew more slowly than nestlings from control nest boxes, indicating a constraining role of high ambient temperatures acting through a change in distribution of resources between growth and thermoregulation. Yet, heat-challenged nestlings in the study by Andreasson et al. (2018) tended to survive better to the next breeding season compared to control birds, providing mixed evidence for the constraining or priming role of developmental temperature on adult phenotype. Furthermore, Page et al. (2022) found that blue tits that were experimentally heated as embryos were more cold tolerant as mature nestlings than nestlings in non-heated nests. The above discrepancies suggest we need to know more about how morphological and physiological responses to developmental temperature affect the capacity for thermal acclimation in a broader range of circumstances, particularly with regards to whether any of the reported phenotypic changes remain in adulthood. To fill this knowledge gap, we must measure the ontogeny and legacy of responses to variation in developmental temperature, including changes in metabolic rate, evaporative cooling capacity and T_b, both in the juvenile stage and in adulthood.

Here, we investigated if development during a simulated heatwave affected offspring thermal, metabolic and hygric physiology, and if any such effects remained in adulthood several weeks after the heatwave had passed. We studied this in Japanese quail (*Coturnix japonica*) that completed the first half of their somatic development under simulated heatwave-like conditions or under normal thermal conditions, and the second half in a common garden. Epigenetic adaptation to temperature appears during critical phases of development (Yahav, 2015). Even though precocial species like Japanese quail are covered with feathers and can thermoregulate from an early age, they are typically not fully homeothermic until some weeks after hatch (Jørgensen & Blix, 1988; Nord & Nilsson, 2021; reviewed by Price and Dzialowski, 2018). Accordingly, incipient thermogenesis develops from late incubation and over the first 10 or so days post-hatch (Yahav, 2015), suggesting there could be lasting effects on the thermoregulatory phenotype if chicks complete the first part of their post-embryonic life in heatwave-like conditions. If

developmental conditions prime heat acclimation, we predicted, under the environmental matching hypothesis, that heatwave birds would have lower T_b and correspondingly lower metabolic heat production rate (MHP) and a higher capacity for evaporative cooling during heat stress (Fig. 1A). However, if high developmental temperature constrains development, we predicted, under the silver spoon hypothesis, that heatwave birds would have higher T_b, higher MHP and lover evaporative cooling capacity during a heat challenge (Fig. 1B). If warm temperatures during the early developmental period permanently modifies temperature tolerance, as shown in studies on poultry (e.g., Arjona et al., 1988; Piestun et al., 2011), we also predicted that these phenotypic traits would remain in adulthood (Figs. 1A, B). If, however, developmental temperature-effects are reversible, we predict that the change in traits caused by thermal acclimation would return to pre-acclimation levels according to the phenotypic flexibility hypothesis (Fig. 1C). We also measured body mass and wing length development, because in line with Bergmann's rule, birds are known to grow larger when it is colder (Ashton, 2002) and smaller when it is warmer (Van Buskirk et al., 2010; Teplitsky et al., 2008). This is expected to decrease or increase dry heat loss rate due to a higher ratio between body surface area and volume. If a higher surface area to volume ratio is biologically meaningful to increase heat transfer rate in the warmth, we predicted that birds growing up under heatwave-like conditions would have lower body mass and longer wings in keeping with established biogeographic rules (Ryding et al., 2021). This study provides new insights into how the developmental period influences how birds cope with increasing temperatures in the short- and long term, by providing data on whether phenotypic effects are lasting, and under which circumstances such responses may be adaptive or maladaptive.

Methods

Species and husbandry

Japanese quail eggs (n=60) were bought from a commercial breeder (Sigvard Månsgård, Åstorp, Sweden) and were incubated in 37.9 ± 0.52°C at 50 % relative humidity using Brinsea OvaEasy 190 incubators (Brinsea, Weston-super-Mare, United Kingdom). The eggs were stored at room temperature (18-20°C) and were turned manually twice daily until put into the incubator (6 eggs per day). On day 15 of incubation, the eggs were moved from the incubation trays to a hatching tray contained inside the incubator. The eggs were checked for signs of hatching daily from day 17 onwards. The incubation period was 19 days (range: 17 to 21d). Forty-five of 60 eggs (75%) hatched.

After hatching, the chicks were left inside the incubator until completely dry or for a maximum of 12 hours. They were then transferred to open pens (310 × 120 × 60 cm) dressed with wood shavings. During the first 3 weeks after hatching, housing temperature was either 20° (henceforth "control treatment") or 30°C to simulate heatwave conditions (henceforth "warm treatment"). From then on, all chicks were

housed in a common garden at 20°C until the end of the experiment. These temperatures were near the upper and lower critical temperatures, respectively, in Japanese quail chicks (Ben-Hamo et al. 2010) and broadly similar to those used by others studying the thermal sensitivity of development in quail (Burness et al., 2013). Chicks were assigned randomly to the warm (n=25) or control (n=20) treatments. The quail had *ad libitum* access to pelleted feed, water, sandbathes and crushed seashells throughout the experiment. Mealworms and vegetables (kale, carrot, or lettuce) were each offered once weekly on alternate days. Until 3 weeks old, the birds were fed turkey starter (Kalkonfoder Start, Lantmännen, Stockholm, Sweden; 25.5% protein). After 3 weeks of age, the feed was switched to turkey grower (Kalkonfoder Tillväxt, Lantmännen, Stockholm, Sweden; 22.5% protein). Until 3 weeks old, all birds had access to a heat lamp providing a temperature of 37°C at floor level, but no heating source was provided from week 3 onwards. Photoperiod was 12:12 LD (lights on from 06:00 to 18:00 GMT+2) throughout the experiment.

Measurements of body size and body temperature

Morphometric measurements were taken starting on day 1 and then once weekly throughout the experiment. On day 1, birds were banded and weighed (± 0.1 g). From day 7 onwards, wing length was also measured (± 0.5 mm). When the birds were 13 to 16 days old, we implanted a temperature-sensitive PIT tag (LifeChip BioTherm, Destron Fearing, South St. Paul, MN, USA), 2.1 × 12 mm in size (< 0.5% of body weight), into the intraperitoneal cavity to measure T_b. Ventral skin surrounding the distal end of the sternum was sterilised (70% EtOH) and anaesthetized topically using 5% lidocaine (EMLA®, Aspen Pharma, Durban, South Africa). Thirty to 45 minutes later, the skin was sterilized again, and a sterile PIT tag was inserted immediately beneath the distal tip of the sternal keel using a sterile 12-gauge syringe. This positioned the PIT tag on top of the liver (Andreas Nord, pers. obs.). The incision was closed using UHU cyanoacrylate (Bolton Adhesives, Rotterdam, Nederland) and covered with antiseptic ointment (1% H_2O_2, LHP, Bioglan AB, Malmö, Sweden).

Measurements of metabolic heat production and evaporative water loss

Metabolic heat production (MHP) and evaporative water loss (EWL) were measured using flow-through respirometry in a climate test chamber (Weiss Umwelttechnik C180, Reiskirchen, Germany). Measurements were taken, firstly, at the end of the heatwave treatment when the birds were 3 weeks old and halfway through the somatic growth phase. Measurements were taken again at 8 weeks, when the birds had reached asymptotic size and started reproducing (i.e., when they were young adults). During the first experimental period, we used 3.3 L glass respirometry chambers, and for the second experimental period 8.0 L glass chambers. The chambers were ventilated with dry (drierite; Sigma-Aldrich, Stockholm, Sweden) atmospheric air at 1.873 – 2.638 L/min (mean ± SE: 2.149 ± 0.013 L/min;

standard temperature and pressure, dry, STPD) during the 3 week measurements and 3.653 – 4.751 L/min (mean ± SE: 4.179 ± 0.013 L/min, STPD) during the 8 week measurements as measured with a FB8 mass flow meter (Sable Systems, Las Vegas, NV, USA). We sub-sampled 308 – 388 ml/min (mean ± SE: 333 ± 1 ml/min, STPD) from the main gas stream using a SS-4 sub-sampler (Sable Systems) for gas analyses. Oxygen was measured using a FC-10 analyser (Sable Systems) and water vapor was measured with a RH-300 water vapour meter (Sable Systems). Water vapour and carbon dioxide were removed from the airstream before measuring oxygen using drierite and ascarite (II) (Acros Organics, Geel, Belgium). The oxygen analyser was zero calibrated using 100% N_2 and span calibrated to 20.95% O_2 using dry (drierite) room air. The RH-300 was zero calibrated using dry room air, and span calibrated by exploiting the dilution of oxygen by water vapour in moist air. Thus, by recording barometric pressure (BNP, kPa) and O_2 content in dry (O_{2dry}) and wet (O_{2wet}) air, it is possible to calculate water vapour pressure (WVP, kPa):

$$\mathrm{WVP} = \mathrm{BP} \times \left(\mathrm{O}_{2_{\mathrm{dry}}} - \mathrm{O}_{2_{\mathrm{wet}}}\right) / \mathrm{O}_{2_{\mathrm{dry}}} \qquad \text{(eq. 1)}$$

Birds were placed on a metal grid platform over a reservoir of mineral oil to avoid that evaporation from faeces affected the measurements of EWL (Lasiewski et al., 1966). Air temperature in the chambers were measured using thermocouples (36-gauge type T, copper-constantan) connected to a thermocouple box (TC-2000, Sable Systems) at a height where the temperature reading was not affected by contact with the bird. T_b was measured at 1 Hz throughout the experiment by placing custom-made antennas connected to a RM310 multiplexer board (Biomark, Boise, ID, USA) below the respirometry chambers. Measurements were performed with the climate test chamber set at 10, 20, 30 and 40°C. The corresponding temperatures in the respirometer chambers were 12 ± 0.14, 21 ± 0.07, 31 ± 0.05 and 40 ± 0.07°C.

All experiments took place during daytime. Birds were placed in a climate chamber illuminated by a light bulb at least 30 minutes before the start of data collection for thermal acclimation to the first measurement temperature (which was always 10°C). During the first experimental period, a measurement cycle started with a 10-minute baseline followed by 10 minute of sequential data collection for each of four birds and ended with a 20-minute baseline. During the second experimental period (i.e., when birds where 8 weeks old), 2 or 3 birds were measured simultaneously. Measurements started with a 15-minute baseline followed by 10 minute of data collection for each bird and ended with a 20-minute baseline. Temperature in the climate chamber was increased acutely to the next target temperature at the start of the last baseline measurement in a cycle, the new target being reached within 10 – 20 min. A bird was removed from the experiment if it showed signs of distress that did not pass in 5 minutes, or if T_b rose > 44°C at the same time as it showed signs of distress.

Data analyses

Missing data

Of the 45 birds that hatched, 39 (control: 17; warm: 22) were subsequently used in the first experimental period and 36 (control: 16; warm: 20) in the second experimental period (Table 1). Four birds died of natural causes or were euthanized before any measurements took place, and an additional 3 died between 3 and 8 weeks of age. Furthermore, 1 bird was never considered for any measurements because it showed abnormally slow growth rate and was subsequently subject to severe pecking by the other birds. One bird was left out of the thermal and metabolic experiments because it could not be fitted into the measurement schedule (see above) but was included in the analyses of morphological characteristics. Five birds were removed from the experiment before measurements in 40°C at 3 weeks of age due to signs of stress (see above). Data from 8 birds from 6 sessions (of 100 total) were excluded from the data set because gas concentrations were not at steady state, typically because the birds were non-resting and so did not conform to the criteria for measuring resting metabolic rate (IUPS Thermal Commission, 2003). An overview of sample sizes per ages and response variable is provided in Table 1.

Calculations

Oxygen consumption (ml / min) was calculated from the most stable 2 min period of the 10 min recording using eq. 11.1 in Lighton (2008), and was converted to metabolic heat production (MHP, in W) assuming 1 ml of O_2 = 20 J (Kleiber, 1961). Evaporative water loss (EWL, in mg / min) was calculated from the same period using eq. 11.9 in Lighton (2008) and was converted to EHL (in W), assuming 2.406 J is required to evaporate 1 ml of H_2O (Wallace and Hobbs, 2006). We then calculated evaporative cooling efficiency as the ratio between EHL and MHP (Lasiewski et al., 1966). Body temperature was calculated as the mean of the 2 min period for which MHP and EWL were defined.

Statistical analyses

Statistical analyses were made in R (version 4.0.3; http://www.R-project.org/) using linear mixed models (lmer function in lme4; Bates et al., 2015). To test if the treatment affected size and growth, we used body mass or wing length as dependent variables, treatment as a factor, and age and age^2 as co-variates. The original model also included the interactions treatment × age and treatment × age^2. Bird ID was used as a random factor to account for repeated measurements on the same individual. For the thermal and metabolic models, MHP, EWL, EHL/MHP, and T_b were used as response variables. To test the effects of measurement temperature on these metrics, we fitted separate models at 3 and 8 weeks of age with treatment, temperature, and treatment × temperature as factors, body mass as a continuous

covariate and bird ID as random intercept (to account for repeated measurements). MHP, EWL and EHL/MHP were log-transformed before the analyses to meet parametric assumptions.

P-values for both fixed and random factors were assessed using likelihood ratio tests. The interaction was removed from the model when non-significant (i.e., $P > 0.05$), but all main effects were retained. If interactions were significant, a post hoc test (using the pairs() function in the emmeans package; Lenth, 2016) was performed between treatments within temperatures. Model estimates where calculated using the emmean() function in the emmeans package (Lenth, 2016), and were back-transformed from the log scale when applicable. Figures show raw data.

Results

Body mass and wing length

Both body mass and wing length increased non-linearly with age, with asymptotic phases for both traits reached between 5 and 6 weeks of age. Neither growth curve differed between the treatments. Nor was there any difference in the body mass (Fig. 2A) or wing length (Fig. 2B) intercepts between warm- and control birds (Table 2). Birds had reached 58% of their adult (i.e., 8 week) body mass, and 81 % of adult wing length at 3 weeks of age (Table 2; Fig. 2).

Effects of air temperature on thermal and metabolic responses

There was no interaction between treatment and temperature on log(MHP) at any age (Table 3). At 3 weeks, control birds had higher log(MHP) (2.09 ± 0.058 W) than warm birds (1.94 ± 0.051 W) (Table 3; Fig. 3A), but there was no effect of treatment on log(MHP) at 8 weeks (Table 3; Fig. 3A). Log(MHP) increased with increasing body mass both at 3 and 8 weeks (Table 3).

Log(EWL) was not affected by the interaction between treatment and temperature, nor by treatment at any age (Table 3). At 3 and 8 weeks, log(EWL) increased with temperature (Table 3; Fig. 3B). Log(EWL) was not affected by body mass at 3 weeks (Table 3), but it increased with body mass at 8 weeks (Table 3).

The log-transformed ratio between EHL and MHP (i.e., log(EHL/MHP)), which measures how large a proportion of MHP that the birds lost by evaporation, was affected by the interaction between treatment and temperature at 3 weeks. Specifically warm birds lost a larger proportion of MHP (0.618 ± 0.022)

than control birds (0.523 ± 0.027) in 40°C (Table 3; Fig. 3C), but there was no difference between the treatments in any other temperature (Table 3). At 8 weeks, log(EHL/MHP) was not influenced by treatment, neither as a main effect or when interacting with temperature (Table 3), but it increased significantly between all experimental temperatures (Table 3; Fig. 3C). At 3 weeks, log(EHL/MHP) decreased with increasing body mass (Table 3) but this effect had disappeared by week 8 (Table 3).

T_b did not differ between treatments at any age, neither when considering the main effect nor its interaction with air temperature (Table 3). However, T_b increased with temperature across treatments for both ages (Table 3; Fig. 4). There was no effect of body mass on T_b at 3 weeks (Table 3). At 8 weeks, T_b increased with increasing body mass (Table 3).

Discussion

By raising Japanese quail in heatwave-like conditions, we found that birds growing up in warm post-hatch environments were more equipped to counter high air temperature as juveniles than control birds. In line with our predictions, warm-reared birds had lower metabolic rate than control birds, and so could dissipate more of their metabolic heat production (MHP) by evaporation compared to control birds when exposed to hot air (i.e., the ratio between evaporative heat loss (EHL) and MHP was higher). Thus, birds growing up warm are more likely to handle heat better, because evaporative cooling capacity is crucial for heat tolerance (McKechnie and Wolf, 2019; McKechnie et al., 2016). Our results contrast a recent study on zebra finches (*Taeniopygia guttata*) that found no effects of temperature on MHP or evaporative water loss (EWL) close to fledging (Ton et al., 2021). However, the zebra finches studied by Ton and colleagues had lower T_b than control birds both at fledging and as juveniles more than a month after the birds had fledged and the experimental heatwave had ended. (Ton et al., 2021). In contrast to this, we found no effects on T_b at any age, and there was also no difference in MHP or evaporative cooling capacity at 8 weeks, when the birds had been kept in common garden for more than one month. This indicates that evaporative cooling capacity develops faster when birds grow up in warmer conditions, but that the temperature stimulus in this study did not cause permanent effects on thermal and hygric physiology.

Even though a smaller body size could be expected according to biogeographical rules, we found no effect of developmental temperature on body mass and wing length in our study. It is possible that temperature-dependent constraints on development are not much of an issue when there is unrestricted access to food and water. This is different from the effects of high temperature in the wild, where heatwave-conditions are often associated with reduced food availability and drought (Kingsolver and

Woods, 1997; Smith, 1982; reviewed by Cunningham et al., 2021). Moreover, while many studies have shown that high temperatures cause decreases in body size (e.g. Salewski et al., 2010, Andrew et al., 2017, Andreasson et al., 2018), there are several exceptions to this pattern (Salewski et al., 2010; Siepielski et al., 2019; Ton et al., 2021). Thus, selection for small body size due to warmer temperatures might not be a general response (Siepielski et al., 2019). Nonetheless, Burness et al. (2013) and Andrew et al. (2017) report on reduced size in heat-challenged captive birds with *ad libitum* access to food and water. Moreover, broilers raised for meat production gain less body mass and eat less food, and have higher mortality, when exposed to both acute (Zaboli et al., 2017; Arjona et al., 1988) and chronic (Imik et al., 2012; Sohail et al., 2012; Ranjan et al., 2019) heat stress. Perhaps the difference between the control and warm treatment in our study was too small, or the warm temperature too low, to cause any irreversible changes on body size.

Alternatively, it is possible that other selection pressures for large body size, such as competitive ability in males or egg size in females, were stronger than selection for any size-related increase in dry heat flux in the warmth; demands for which would be more easily accommodated by physiological flexibility within a generation. Future studies should address if developmental temperature affected size of the appendages. According to Allen's rule (Allen, 1877), appendages such as the legs and bills of birds or the tails and ears of mammals, should be longer in warmer temperatures (cf. Demicka & Caputa, 1993). Since bird bills and legs are both richly vascularised and uninsulated, changes in local circulation can quickly either increase or decrease heat flux (Scholander et al., 1950; Tattersall et al., 2009). Thus, long appendages are likely aiding thermoregulation by the whole bird much more than any change in size per se.

The environmental matching hypothesis predicts that animals growing up in warmer environments should be better at handling warm temperatures as adults, which is shown by several studies (e.g., Demicka and Caputa, 1993; Stawski and Geiser, 2020; reviewed by Nord and Giroud, 2020). In other studies, silver spoon effects appear to be more common, reflected by reduced thermoregulatory performance or constrained growth after development in thermally challenging conditions (reviewed by Andreasson et al., 2020). We found support for neither hypothesis. However, it is important to note that we did not measure maximum heat and cold tolerance, which might be more telling of thermal acclimation than submaximal physiological rates. Such measurements could also be more informative when extrapolating laboratory results to free-ranging animals because, in the wild, temperatures vary by several tens of degrees between different microhabitats, e.g., in relation to vegetation and topography (Carroll et al., 2016; Cunningham et al., 2015; Cunningham et al., 2021), suggesting knowledge of heat

tolerances will inform on which animals are better able to exploit resources spread across the thermal landscape.

Conclusion

We show that warm temperatures early in life can make chicks more prepared to counter heat stress. However, we found no support for environmental matching of juvenile and adult environments, because reproductively mature warm-origin birds that had spent the latter half of their growth and maturation period in a common garden thermoregulated in the same way as control birds. Thus, a mild or short-duration heatwave during the developmental period does not seem to be constraining for growth and does not seem to have any obvious consequences for how animals respond to cold or warm temperatures as adults. Future studies should address if a longer heatwave period, or a more severe heat treatment, cause longer-term or irreversible effects. Such studies should also address if there are any differences between appendages of birds subjected to different temperatures during development.

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Ethics

Ethical approval for the experiment was granted by the Malmö/Lund Animal Ethics Committee (permit no. 9246-19).

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TABLES

Table 1. The number of Japanese quail used when studying the thermo-physiological responses (metabolic heat production (MHP), evaporative water loss (EWL), body temperature (T_b)) to increasing air temperature at two different ages. The birds were raised in 20°C (control) or 30°C (warm) for the first 3 weeks of life, and in 20°C thereafter. The same birds were measured at both ages and at all temperatures, but some experiments were excluded from the final data set for reasons stated in the main text.

		Sample size			
	Total	*10°C*	*20°C*	*30°C*	*40°C*
3 weeks	39				
Control		17	17	17	15
Warm		22	22	22	19
8 weeks	36				
Control		16	15	16	15
Warm		18	19	19	18

Table 2. Estimates, test statistics, degrees of freedom, P-values and standard deviations (σ) for the random factor for models explaining the effects of developing in either control (20°C) or warm (30°C) temperature conditions on body mass and wing length in Japanese quail.

Model	**Estimate ± SE**	**LR**	**Df**	**P**	σ_{ID} | σ_{total}
Body mass (g)					
Treatment		0.19	1	0.6601	
Age	35.07 ± 0.720	476.71	1	<0.0001 ***	
Age^2	-2.75 ± 0.078	225.62	1	<0.0001***	
Treatment:Age		0.30	1	0.5870	
Treatment:Age^2		0.39	1	0.5352	
Random factor bird ID		67.01	1	<0.0001 ***	11.44|25.69
Wing length (mm)					
Treatment		2.07	1	0.1500	
Age	58.40 ± 1.497	560.34	1	<0.0001 ***	
Age^2	-2.83 ± 0.145	429.19	1	<0.0001 ***	
Treatment:Age		0.01	1	0.9417	
Treatment:Age^2		0.00	1	0.9776	
Random factor bird ID		0.08	1	0.7844	0.60|5.99

566 **Table 3.** Estimates, test statistics, degrees of freedom, P-values and standard deviations (σ) for the
567 random factor for models explaining the effects of developing in either control (20°C) or warm (30°C)
568 temperature conditions on treatment at 3- and 8 weeks on metabolic heat production, evaporative
569 water loss, evaporative heat loss/metabolic heat production and body temperature in Japanese
570 quail. All dependent variables were log-transformed in the models, but back-transformed model
571 estimates are shown in the table. Statistics for main effects is not provided when the interaction
572 between treatment and temperature was significant. Different letters within brackets represent
573 significant (P < 0.05) post hoc comparisons. Abbreviations: MHP: Metabolic heat production; EWL:
574 Evaporative water loss; EHL/MHP: Evaporative heat loss/Metabolic heat production; T_b: Body
575 temperature.

Model	Estimate ± SE	LR	Df	P	σ_{ID} | σ_{total}
Log(MHP (W)) – 3 weeks					
Treatment		3.88	1	0.0460 *	
Control [A]	2.03 ± 0.060				
Warm [B]	1.88 ± 0.049				
Temperature		187.06	3	<0.0001 ***	
10°C [A]	2.51 ± 0.061				
20°C [B]	2.01 ± 0.049				
30°C [C]	1.51 ± 0.037				
40°C [B]	1.92 ± 0.049				
Treatment:Temperature		4.07	3	0.2536	
Body mass	1.004 ± 1.001	8.14	1	0.0043 **	
Random factor bird ID		45.15	1	<0.0001 ***	1.121.12|2.23
Log(MHP (W)) – 8 weeks					
Treatment		1.21	1	0.2711	
Temperature		75.27	3	<0.0001 ***	
10°C [A]	2.93 ± 0.097				
20°C [BD]	2.35 ± 0.078				
30°C [CD]	2.01 ± 0.066				
40°C [D]	2.19 ± 0.073				
Treatment:Temperature		2.02	3	0.5677	
Body mass	1.002 ± 1.001	5.42	1	0.0199 *	
Random factor bird ID		14.91	1	0.0001 ***	1.12|2.29
Log(EWL (g h^{-1})) – 3 weeks					
Treatment		0.00	1	0.9516	
Temperature		398.38	3	<0.0001 ***	
10°C [A]	0.500 ± 0.014				
20°C [B]	0.583 ± 0.016				
30°C [C]	0.710 ± 0.020				
40°C [D]	1.632 ± 0.047				
Treatment:Temperature		3.04	3	0.3856	
Body mass		0.68	1	0.4085	
Random factor bird ID		83.35	1	<0.0001 ***	1.16|2.25
Log(EWL (g h^{-1})) – 8 weeks					
Treatment		2.01	1	0.1559	
Temperature		321.04	3	<0.0001 ***	
10°C [A]	0.556 ± 0.019				
20°C [AB]	0.575 ± 0.020				
30°C [B]	0.628 ± 0.021				
40°C [D]	2.036 ± 0.070				
Treatment:Temperature		1.35	3	0.7173	
Body mass	1.002 ± 1.001	5.28	1	0.0216 *	
Random factor bird ID		25.56	1	<0.0001 ***	1.14|2.30
Log(EHL/MHP) – 3 weeks					

Treatment:Temperature		8.36	3	0.0391 *	
10°C					
Control [A]	0.134 ± 0.005				
Warm [A]	0.134 ± 0.004				
20°C					
Control [A]	0.187 ± 0.007				
Warm [A]	0.202 ± 0.007				
30°C					
Control [A]	0.299 ± 0.011				
Warm [A]	0.328 ± 0.011				
40°C					
Control [A]	0.518 ± 0.020				
Warm [B]	0.612 ± 0.021				
Body mass	0.998 ± 1.001	4.57	1	0.0325 *	
Random factor bird ID		17.77	1	<0.0001 ***	1.09|2.23
Log(EHL/MHP) – 8 weeks					
Treatment		0.14	1	0.7130	
Temperature		318.56	3	<0.0001 ***	
10°C [A]	0.127 ± 0.005				
20°C [B]	0.163 ± 0.006				
30°C [C]	0.209 ± 0.007				
40°C [D]	0.623 ± 0.022				
Treatment:Temperature		0.91	3	0.8238	
Body mass		0.02	1	0.8748	
Random factor bird ID		3.67	1	<0.0554	1.09|2.30
T_b (°C) – 3 weeks					
Treatment		0.61	1	0.4359	
Temperature		218.04	3	<0.0001 ***	
10°C [A]	42.68 ± 0.061				
20°C [B]	42.31 ± 0.061				
30°C [C]	42.00 ± 0.061				
40°C [D]	43.61 ± 0.064				
Treatment:Temperature		4.17	3	0.2491	
Body mass		0.01	1	0.9046	
Random factor bird ID		30.35	1	<0.0001 ***	1.23|2.61
T_b (°C) – 8 weeks					
Treatment		0.37	1	0.5409	
Temperature		134.80	3	<0.0001 ***	
10°C [A]	43.07 ± 0.077				
20°C [B]	42.66 ± 0.077				
30°C [B]	42.57 ± 0.076				
40°C [C]	43.69 ± 0.078				
Treatment:Temperature		2.97	3	0.3960	
Body mass	0.005 ± 0.003	4.73	1	0.0297 *	
Random factor bird ID		43.69	1	<0.0001 ***	1.40|2.75

FIGURES

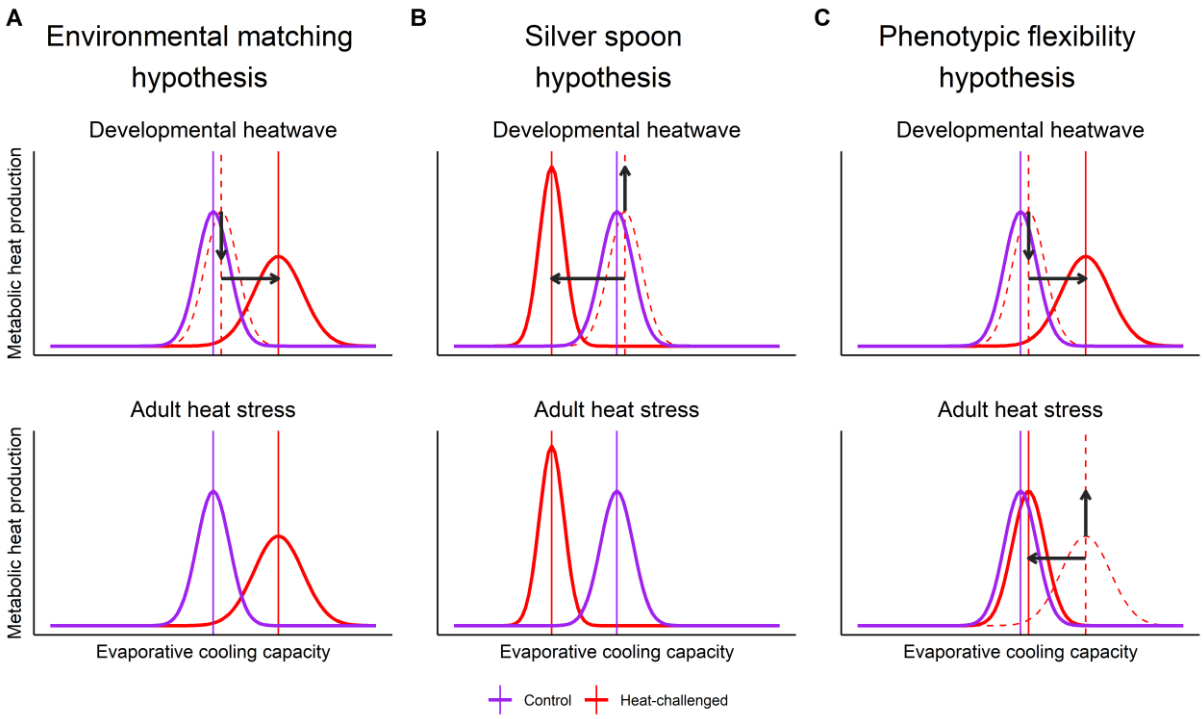
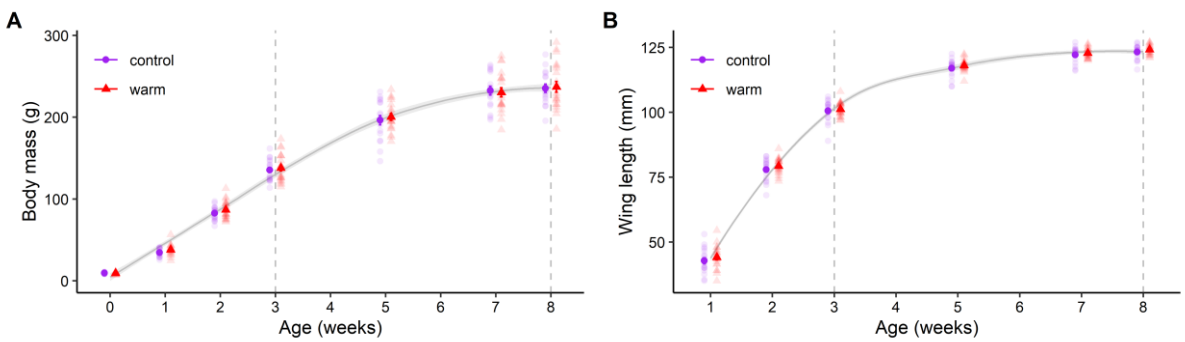
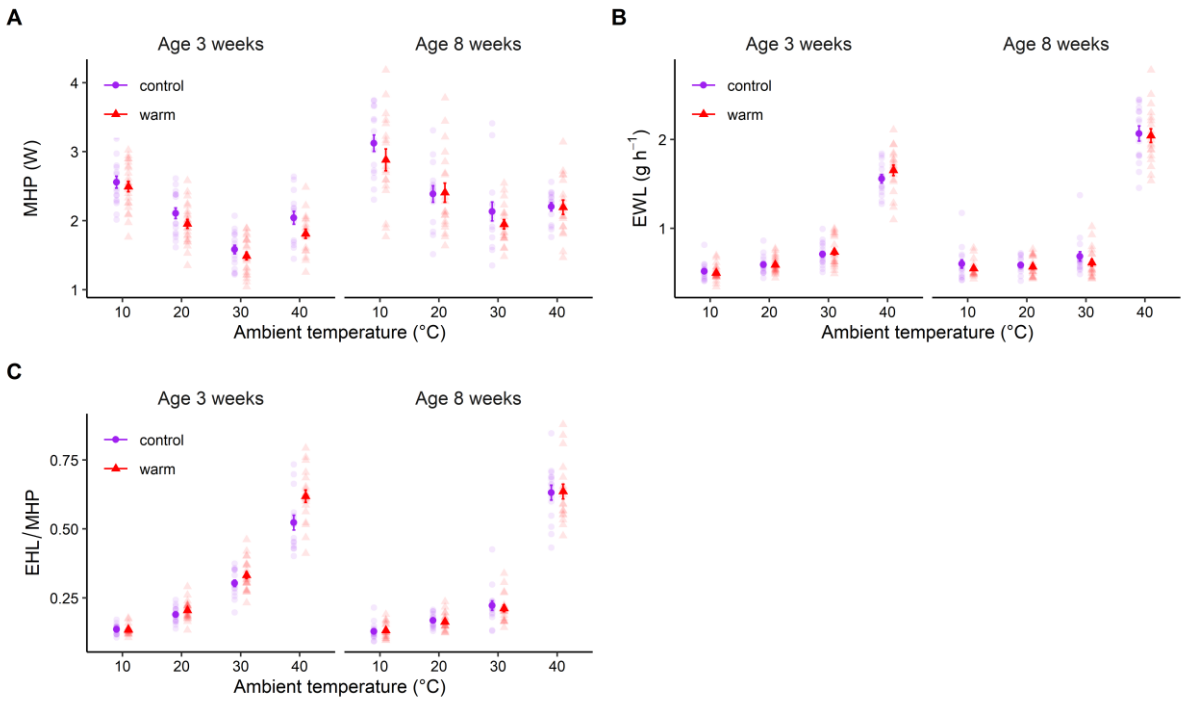


Figure 1. Three hypothetical scenarios for thermo-physiological changes in response to a developmental heatwave. A) Under the Environmental matching hypothesis, a developmental heatwave leads to a non-reversible reduction in metabolic heat production and improvement in evaporative cooling capacity, such that individuals developing under heatwave-like conditions are more heat tolerant as adults. B) Under the Silver spoon hypothesis, a developmental heatwave impairs normal growth and maturation of the thermoregulatory system which lowers heat tolerance both in juveniles and adults. C) Under the Phenotypic flexibility hypothesis, a developmental heatwave triggers reduced metabolic heat production and improved evaporative cooling capacity. However, these effects are reversible so that the developmental thermal environment does not affect heat tolerance in adulthood.



592 **Figure 2. Effects on body mass and wing length when Japanese quail developed in either control**
593 **(20°C) or warm (30°C) temperature conditions.** The figure shows mean ± SE (A) body mass and (B)
594 wing length of Japanese quail during the 8-week experimental period. The grey curve is LOESS (locally
595 estimated scatterplot smoothing) ± 95% confidence interval. The dashed lines represent the timing of
596 measurements of metabolic rate and evaporative water loss. Sample sizes are stated in the Methods.
597 Transparent points show raw data.

598



599

600 **Figure 3. Effects on metabolic heat production (MHP), evaporative water loss (EWL) and the ratio**
601 **between evaporative heat loss (EHL) and MHP when Japanese quail developed in either control**
602 **(20°C) or warm (30°C) temperature conditions.** The figure shows mean ± SE (A) MHP, (B) EWL and
603 (C) EHL/MHP of Japanese quail in the different test temperatures (10°C, 20°C, 30°C and 40°C) at 3
604 weeks and 8 weeks of age. Sample sizes are stated in the Methods. Transparent points show raw
605 data.

606

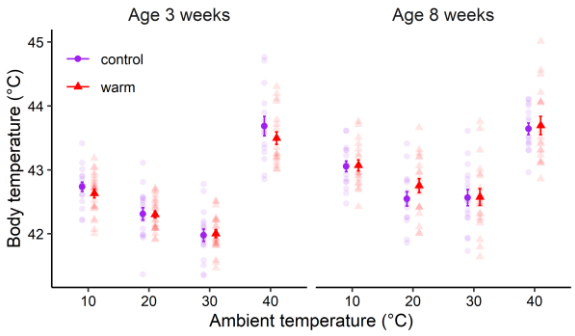


Figure 4. Effects on body temperature (T_b) when Japanese quail developed in either control (20°C) or warm (30°C) temperature conditions. The figure shows mean ± SE T_b of Japanese quail in the different test temperatures (10°C, 20°C, 30°C and 40°C) at 3 weeks and 8 weeks of age. Sample sizes are stated in the Methods. Transparent points show raw data.