

A prenatal acoustic signal of heat affects thermoregulation capacities at adulthood in an arid-adapted bird.

Anaïs Pessato^{1*}

Andrew E. McKechnie^{2,3}

Mylene M. Mariette^{1,4*}

¹Centre for Integrative Ecology, School of Life & Environmental Sciences, Deakin University, Geelong 3216, Australia.

²South African Research Chair in Conservation Physiology, South African National Biodiversity Institute, Pretoria 0001, South Africa

³DSI-NRF Centre of Excellence at the FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0001, South Africa

⁴Doñana Biological Station EBD-CSIC, 41092 Seville, Spain

*Corresponding authors

apessato@deakin.edu.au

m.mariette@deakin.edu.au

Supplementary information

Supplementary materials and methods

Experience from hatching to test at adulthood

After hatching, nestlings were returned to either their parents or foster parents in mixed broods (n=18 birds) or single prenatal playback broods (n=16 birds) in shared outdoor aviaries. Mixed broods allow controlling for shared postnatal environment but cannot occur naturally; incubating parents cannot signal to only half of the clutch and therefore would not normally rear mixed broods). Before sexual maturity, offspring (both control and treatment groups) were moved together to another aviary where they all experienced natural weather conditions (from 2014 to 2018: mean min temperature= 4.7°C, mean max temperature=27.6°C, weather data obtained from Bureau of Meteorology, www.bom.gov.au). There was no difference in survival between treatment and control groups at early stages¹.

At adulthood, birds were tested during daytime, when they would normally experience high air temperatures. To lower the possible stress due to social isolation in the metabolic chamber during the trial, familiar acoustic background from birds in the holding room was broadcast (as routinely done in behavioural experiments² with a Bluetooth speaker (QUDO Splashproof Bluetooth Speaker, officeworks LTD, Australia).

Open flow-through respirometry system and calibration of equipment

We used a pump (Gast DAA-V515-ED, Cole-Parmer) to push dry air (scrubbed with silica gel and Drierite ®) into the metabolic chamber (1.5 L, plastic clip container, 8 x 18 x 11.5 cm). The air in the metabolic chamber was maintained with at low humidity level by regulating the airflow with a mass flow controller (Alicat scientific Inc., USA). Mass flow controller was calibrated and accurate within 0.008 SPLM within the range we used (1 to 3.5 L.min⁻¹). Baseline air was subsampled between each T_a stage for five minutes. Baseline and excurrent

chamber air were subsampled and pulled by a pump (SS4 subsampler, Sable Systems) at 213 mL.min⁻¹ through the H₂O analyser (RH-300, Sable Systems) and CO₂ analyser (CA-10, Sable Systems). H₂O analyser was calibrated daily using the O₂ dilution technique³ and the CO₂ analyser every second day using certified gas with a known CO₂ concentration of 2080 ppm (BOC, Australia). Both of the analysers were zeroed with pure nitrogen (Nitrogen 5.0; 99.999%, Supagas, Australia). Gas concentrations and temperatures were recorded via an analog-digital converter (UI2, Sable Systems) and Expedata software (Sable Systems).

During the trial, body temperature was recorded every 10 sec using a PIT tag reader (HPR plus, Biomark, Boise ID, 172 USA). No aberrant values were recorded; all values during the one-minute measure were thus averaged. A subset of PIT tags were calibrated in a water bath against a type T thermocouple (BAT-12, Physitemp Instruments Inc., USA) at water temperature ranging from 40°C to 48°C. PIT tags were accurate within $0.27 \pm 0.03^\circ\text{C}$.

Data processing and statistical analyses

Relative water economy (RWE) was computed as MWP/EWL with MWP calculated from rates of CO₂ production assuming 0.801 mg H₂O mL⁻¹ CO₂⁴. We also computed the evaporative cooling capacity (EHL/MHP) as EHL/MR. We converted rates of EWL to heat loss (EHL in W) assuming a latent heat of vaporisation of 2.4 J mg⁻¹ H₂O⁵.

In all analyses, we did not include clutch or brood ID because the variance for clutch or brood ID was often close to zero. This is likely because over half of the individuals in this experiment did not have siblings (20 individuals without genetic siblings out of 26 clutches, and 14 individuals without foster siblings out of 22 broods), due to normal mortality of embryos, hatchlings and juveniles (as well as adult mortality).

Supplementary tables

	Max T _a reached			Complete trial		
<i>Predictors</i>	<i>Est.</i>	<i>SE</i>	<i>p</i>	<i>Est.</i>	<i>SE</i>	<i>p</i>
Reduced model						
Intercept	17.544	8.997	0.051	-12.181	6.796	0.073
Playback	13.654	7.540	0.070	-3.057	3.755	0.416
Mass _{init}	-0.076	1.891	0.968	-1.715	1.820	0.346
Time	4.256	3.180	0.181	-4.595	3.046	0.132
Trial	-3.747	3.343	0.262	3.572	2.745	0.193
Sex	-1.833	3.866	0.636	0.337	3.760	0.929
Playback x time	-18.718	9.062	0.039			
Full model						
Intercept	17.544	8.997	0.051	-12.163	7.513	0.105
Playback	13.654	7.540	0.070	-3.052	3.795	0.421
Mass _{init}	-0.076	1.891	0.968	-1.714	1.861	0.357
Time	4.256	3.180	0.181	-4.587	3.567	0.198
Trial	-3.747	3.343	0.262	3.564	3.359	0.289
Sex	-1.833	3.866	0.636	0.334	3.769	0.929
Playback x time	-18.718	9.062	0.039	0.001	4.885	1

Table S1: Outputs of the mixed effect models on heat tolerance variables: maximum T_a reached (42 or 44°C, considering all birds, n=67) and the ability to complete trial (i.e. staid 20 minutes at T_a=44°C) among birds reaching T_a=44°C (n=53). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

	Mass loss			Mass recovery post-trial		
<i>Predictors</i>	<i>Est.</i>	<i>SE</i>	<i>p</i>	<i>Est.</i>	<i>SE</i>	<i>p</i>
<i>Reduced model</i>						
Intercept	0.576	0.073	<0.001	55.301	20.472	0.009
Playback	-0.045	0.062	0.475	42.007	17.436	0.020
Mass _{init}	0.045	0.028	0.108	-16.049	7.162	0.030
Time	-0.061	0.029	0.041	5.050	12.373	0.686
Trial	-0.001	0.030	0.968	18.047	9.748	0.073
Sex	0.085	0.062	0.180	16.447	14.748	0.273
Playback x time				-64.761	18.659	0.002
<i>Full Model</i>						
Intercept	0.565	0.073	<0.001	55.301	20.472	0.009
Playback	-0.009	0.069	0.901	42.007	17.436	0.020
Mass _{init}	0.046	0.028	0.100	-16.049	7.162	0.030
Time	-0.029	0.038	0.452	5.050	12.373	0.686
Trial	-0.005	0.030	0.560	18.047	9.748	0.073
Sex	0.086	0.062	0.176	16.447	14.748	0.273
Playback x time	-0.072	0.056	0.208	-64.761	18.659	0.002

Table S2: Outputs of mixed effect models on mass loss (n=67 for 34 birds) and mass recovery post-trial (n=66 for 34 birds). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

	<i>Sqrt activity_{stage} (3-minutes)</i>							
	Above inflection pt		Max T_a reached		T_a=35°C		T_a=27°C	
<i>Predictors</i>	<i>Est.±SE</i>	<i>p</i>	<i>Est.±SE</i>	<i>p</i>	<i>Est.±SE</i>	<i>p</i>	<i>Est.±SE</i>	<i>p</i>
Reduced model								
Intercept	1.184±0.131	<0.001	1.733±0.233	<0.001	0.560±0.138	<0.001	0.301±0.135	0.029
Playback	0.002±0.104	0.985	0.153±0.144	0.296	-0.018±0.089	0.838	-0.045±0.086	0.600
Mass _{init}	0.031±0.049	0.535	-0.046±0.069	0.501	0.008±0.044	0.862	0.095±0.043	0.032
Time	0.019±0.056	0.740	0.002±0.094	0.984	-0.010±0.068	0.0889	0.150±0.067	0.032
Trial	-0.126±0.059	0.039	-0.212±0.095	0.035	-0.097±0.070	0.173	-0.040±0.068	0.565
Sex	0.014±0.104	0.895	-0.123±0.146	0.408	-0.080±0.089	0.378	0.120±0.086	0.173
T _a	0.279±0.037	<0.001	-0.033±0.155	0.834	/	/	/	/
Playb x Time	0.132±0.056	0.021						
Full model								
Intercept	1.184±0.131	<0.001	0.132±0.056	0.021	0.598±0.141	<0.001	0.265±0.132	0.050
Playback	0.002±0.104	0.985	0.0201±0.173	0.251	-0.044±0.113	0.699	0.083±0.107	0.444
Mass _{init}	0.031±0.049	0.535	-0.046±0.069	0.509	0.008±0.044	0.858	0.099±0.043	0.026
Time	0.019±0.056	0.740	0.045±0.129	0.729	-0.032±0.093	0.730	0.262±0.085	0.004
Trial	-0.126±0.059	0.039	-0.209±0.097	0.040	-0.095±0.071	0.193	-0.055±0.066	0.411
Sex	0.014±0.104	0.895	-0.123±0.147	0.411	-0.080±0.089	0.375	0.122±0.086	0.165
T _a	0.279±0.037	<0.001	-0.049±0.158	0.759	/	/	/	/
Playb x Time	0.132±0.056	0.021	-0.094±0.190	0.625	0.051±0.138	0.717	-0.253±0.127	0.056

Table S3: Outputs of mixed effect models on activity_{stage} (during the 3 first minutes at a T_a stage) on all birds tested (unless the stage lasted <3min before trial termination): above inflection point (n=146 observations for 34 birds), at max T_a reached (n=55 for activity_{stage}), T_a=35°C and T_a=27°C (n=67). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

Sqrt activity_{42-end}						
<i>Predictors</i>	<i>Reduced model</i>			<i>Full model</i>		
	<i>Est.</i>	<i>SE</i>	<i>p</i>	<i>Est.</i>	<i>SE</i>	<i>p</i>
Intercept	1.039	0.247	<0.001	0.995	0.256	<0.001
Playback	0.184	0.160	0.260	0.261	0.191	0.179
Mass _{init}	0.048	0.079	0.547	0.046	0.079	0.565
Time	0.143	0.110	0.209	0.218	0.154	0.171
Trial	-0.0156	0.116	0.192	-0.153	0.119	0.210
Sex	0.021	0.159	0.897	0.018	0.157	0.911
Playb x Time				-0.164	0.222	0.468

Table S4: Outputs of mixed effect models on activity_{42-end} (during the last 3 minutes at a T_a=42°C, on all birds reaching T_a=44°C, n=53). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

Predictors	Activity _{meas}					
	Max T _a reached		T _a =35°C		T _a =27°C	
	Est.±SE	<i>p</i>	Est.±SE	<i>p</i>	Est.±SE	<i>p</i>
Reduced model						
Intercept	2.792±0.624	<0.001	0.208±0.057	<0.001	0.625±0.074	<0.001
Playback	0.201±0.257	0.447	-0.005±0.035	0.879	-0.055±0.049	0.273
Mass _{init}	-0.066±0.126	0.604	0.025±0.018	0.173	0.012±0.024	0.628
Time	0.095±0.233	0.689	-0.024±0.028	0.412	-0.006±0.036	0.864
Trial	-0.706±0.235	0.009	-0.015±0.029	0.616	-0.203±0.037	<0.001
Sex	-0.008±0.272	0.976	0.014±0.035	0.693	-0.010±0.049	0.842
T _a	-0.433±0.403	0.299	/	/	/	/
Full model						
Intercept	2.626±0.642	<0.001	0.213±0.058	<0.001	0.626±0.076	<0.001
Playback	0.390±0.314	0.226	-0.022±0.046	0.641	-0.055±0.061	0.372
Mass _{init}	-0.063±0.125	0.620	0.025±0.018	0.175	0.012±0.024	0.634
Time	0.309±0.310	0.341	-0.038±0.039	0.335	-0.007±0.049	0.892
Trial	-0.647±0.241	0.018	-0.013±0.030	0.664	-0.203±0.038	<0.001
Sex	-0.046±0.273	0.868	0.014±0.035	0.700	-0.010±0.049	0.841
T _a	-0.427±0.401	0.303	/	/	/	/
Playb x Time	-0.507±0.485	0.315	0.032±0.058	0.587	0.001±0.073	0.989

Table S5: Outputs of mixed effect models on activity_{meas} (10 minutes before and during stable thermoregulatory measurements) at max T_a reached among the calm individuals from which we obtained thermoregulatory values (n=32 for activity_{meas}), T_a=35°C and T_a=27°C (n=67). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

Predictors	MR		EWL		T _b		RWE	
	Est. ± SE	p	Est. ± SE	p			Est. ± SE	p
<i>Reduced model above inflection points</i>								
Intercept	0.271±0.027	< 0.001	9.200±0.456	< 0.001	43.697 ±0.209	< 0.001	0.051±0.004	< 0.001
Playback	0.024±0.014	0.088	0.124±0.269	0.648	0.030±0.167	0.860	0.002±0.002	0.286
Mass _{init}	0.007±0.007	0.346	0.257±0.137	0.071	-0.031±0.075	0.685	<0.001±0.001	0.986
Time	0.005±0.014	0.732	-0.230±0.217	0.318	0.171±0.079	0.042	0.003±0.002	0.097
Trial	0.003±0.014	0.852	-0.489±0.233	0.042	-0.113±0.090	0.218	0.004±0.002	0.023
Sex	0.008±0.014	0.561	0.175±0.272	0.525	-0.218±0.169	0.208	<0.001±0.002	0.919
T _a	0.012±0.008	0.138	1.120±0.134	< 0.001	0.380±0.050	< 0.001	-0.004±0.001	< 0.001
Activity _{meas}	0.030±0.007	< 0.001	0.283±0.125	0.027	0.336±0.056	< 0.001	0.004±0.001	< 0.001
Playb x T _a	0.040±0.012	0.002	0.396±0.191	0.044				
<i>Full model above inflection points</i>								
Intercept	0.271±0.027	< 0.001	9.200±0.456	< 0.001	43.690 ±0.207	< 0.001	0.051±0.004	< 0.001
Playback	0.024±0.014	0.088	0.124±0.269	0.648	0.038±0.166	0.820	0.003±0.002	0.236
Mass _{init}	0.007±0.007	0.346	0.257±0.137	0.071	-0.036±0.075	0.631	<-0.001±0.001	0.958
Time	0.005±0.014	0.732	-0.230±0.217	0.318	0.173±0.078	0.037	0.003±0.002	0.078
Trial	0.003±0.014	0.852	-0.489±0.233	0.042	-0.112±0.089	0.217	0.004±0.002	0.021
Sex	0.008±0.014	0.561	0.175±0.272	0.525	-0.221±0.168	0.200	0.001±0.002	0.790
T _a	0.012±0.008	0.138	1.120±0.134	< 0.001	0.335±0.057	< 0.001	-0.005±0.001	< 0.001
Activity _{meas}	0.030±0.007	< 0.001	0.283±0.125	0.027	0.330±0.055	< 0.001	0.004±0.001	< 0.001
Playb. x T _a	0.040±0.012	0.002	0.396±0.191	0.044	0.120±0.073	0.110	0.003±0.002	0.107

Table S6: Outputs of linear mixed models above T_a inflection points (i.e. corresponding mainly to the three T_a stages at 40°C and above) for metabolic rate (i.e. MR, n=83 observations for 32 birds), evaporative water loss (i.e. EWL, n=83 observations for 32 birds), body temperature (i.e. T_b, n=94 observations for 32 birds) and relative water economy (i.e. RWE, n=83 observations for 32 birds) of calm birds (i.e. activity_{meas}≤3). Bold font corresponds to significant effect (p<0.05). The reference is the control group for playback, morning for time-of-day, here “Time”, trial 1 for trial and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

<i>Predictors</i>	<i>Est. ± SE</i>	<i>p</i>	<i>Est. ± SE</i>	<i>p</i>	<i>Est. ± SE</i>	<i>p</i>	<i>Est. ± SE</i>	<i>p</i>
	MR		EWL		RWE		T_b	
Max T_a (42°C or 44°C)								
Intercept	0.247±0.092	0.013	9.497±1.201	<0.001	0.049±0.009	<0.001	44.085±0.489	<0.001
Playback	0.048±0.042	0.268	0.281±0.551	0.615	0.004±0.004	0.307	0.063±0.229	0.785
Mass	0.018±0.016	0.282	0.225±0.218	0.316	0.003±0.002	0.097	-0.018±0.095	0.854
Time	0.006±0.044	0.900	-0.330±0.568	0.572	0.003±0.004	0.415	0.074±0.207	0.726
Trial	0.027±0.038	0.480	-1.011±0.490	0.056	0.010±0.003	0.012	-0.231±0.185	0.230
Activity _{meas}	0.023±0.022	0.313	0.251±0.292	0.399	0.003±0.002	0.182	0.391±0.119	0.003
Sex	0.017±0.034	0.631	0.300±0.461	0.527	0.001±0.004	0.749	-0.603±0.209	0.013
T _a	0.015±0.052	0.775	2.230±0.695	0.006	-0.013±0.005	0.037	0.502±0.309	0.125
Playb. x time	0.038±0.067	0.576	0.826±0.855	0.353	0.002±0.006	0.693	0.304±0.311	0.349
Playb.x act _{meas}	0.076±0.036	0.046	0.474±0.465	0.320	0.007±0.003	0.044	0.055±0.177	0.760
T_a = 35°C								
Intercept	0.218±0.018	<0.001	0.230±0.431	<0.001	0.181±0.019	<0.001	41.073±0.270	<0.001
Playback	0.005±0.017	0.751	0.153±0.409	0.709	-0.001±0.018	0.941	0.050±0.273	0.855
Mass	0.016±0.007	0.020	0.296±0.164	0.076	-0.003±0.007	0.722	0.152±0.098	0.127
Time	-0.005±0.009	0.554	-0.336±0.222	0.142	0.024±0.09	0.016	0.039±0.115	0.736
Trial	-0.002±0.007	0.789	-0.092±0.174	0.602	0.003±0.007	0.677	0.132±0.091	0.154
Activity _{meas}	-0.007±0.006	0.274	-0.181±0.152	0.240	0.002±0.006	0.750	-0.020±0.078	0.804
Sex	0.012±0.015	0.435	0.798±0.372	0.041	-0.034±0.016	0.044	0.012±0.258	0.962
Playb. x time	0.001±0.013	0.915	-0.204±0.327	0.539	-0.004±0.014	0.785	-0.059±0.163	0.722
Playb.x act _{meas}	<-0.001±0.009	0.998	0.285±0.233	0.229	-0.010±0.010	0.299	0.053±0.121	0.664
T_a = 27°C								
Intercept	0.367±0.024	<0.001	3.099±0.324	<0.001	0.204±0.030	<0.001	41.879±0.369	<0.001
Playback	0.010±0.021	0.649	-0.318±0.276	0.256	0.034±0.021	0.113	0.246±0.326	0.454
Mass	0.020±0.008	0.018	0.133±0.110	0.230	-0.001±0.009	0.928	0.176±0.126	0.168
Time	-0.007±0.010	0.476	-0.061±0.141	0.670	0.006±0.016	0.718	0.127±0.155	0.421
Trial	-0.007±0.010	0.473	-0.425±0.147	0.006	0.039±0.015	0.014	-0.438±0.164	0.011
Activity _{meas}	0.010±0.007	0.171	0.073±0.098	0.460	-0.001±0.010	0.945	0.199±0.109	0.076
Sex	0.002±0.019	0.905	0.433±0.252	0.096	-0.031±0.017	0.090	-0.115±0.296	0.701
Playb. x time	-0.013±0.014	0.376	-0.362±0.210	0.097	0.028±0.024	0.250	-0.322±0.238	0.186
Playb.x act _{meas}	-0.003±0.011	0.805	-0.291±0.156	0.068	0.008±0.015	0.602	-0.115±0.182	0.532

Table S7: Outputs of the full linear mixed-effects models on metabolic rate (MR), evaporative water loss (EWL), relative water economy (RWE) and T_b of calm birds (i.e. activity_{meas} ≤ 3) at the maximal T_a reached (i.e. T_a=42°C or 44°C, n=32 observations for 19 birds), within the thermoneutral zone (T_a=35°C, n=67 for 34 birds) and at mild temperature (T_a=27°C, n=677 for 34 birds). The reference is the control group for playback, morning for time-of-day (here “Time”), trial 1 for trial, and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05).

EHL/MHP	Predictors	Reduced model			Full model		
		Est.	SE	p	Est.	SE	p
Above inflection point*	Intercept	0.923	0.045	< 0.001	0.925	0.045	< 0.001
	Playback	-0.013	0.035	0.714	-0.014	0.035	0.693
	Mass _{init}	0.007	0.015	0.653	0.008	0.016	0.650
	Time	-0.058	0.019	0.006	-0.059	0.019	0.005
	Trial	-0.036	0.020	0.081	-0.037	0.020	0.082
	Sex	0.057	0.035	0.117	0.056	0.035	0.124
	T _a	0.340	0.012	< 0.001	0.350	0.014	< 0.001
	Activity _{meas}	-0.044	0.012	< 0.001	-0.044	0.012	0.001
Playb. x T _a				-0.022	0.017	0.199	
Max T_a (42°C or 44°C)	Intercept	1.385	0.182	< 0.001	1.366	0.186	< 0.001
	Playback	-0.104	0.069	0.164	-0.071	0.086	0.415
	Mass _{init}	-0.053	0.034	0.145	-0.052	0.034	0.152
	Time	-0.066	0.063	0.326	-0.029	0.085	0.743
	Trial	-0.183	0.073	0.025	-0.179	0.074	0.034
	Activity _{meas}	-0.054	0.044	0.231	-0.060	0.045	0.201
	Sex	0.001	0.072	0.990	-0.005	0.074	0.946
	T _a	0.244	0.109	0.047	0.241	0.110	0.052
	Playb. x time				-0.085	0.129	0.530
Playb.x act _{meas}	-0.160	0.070	0.037	-0.158	0.071	0.042	
T_a=35°C	Intercept	0.422	0.053	< 0.001	0.419	0.053	< 0.001
	Playback	0.002	0.042	0.962	0.019	0.048	0.696
	Mass _{init}	0.019	0.020	0.330	0.020	0.020	0.316
	Time	-0.066	0.022	0.007	-0.053	0.030	0.088
	Trial	-0.008	0.023	0.742	-0.010	0.023	0.670
	Activity _{meas}	0.006	0.015	0.699	-0.008	0.020	0.680
	Sex	0.105	0.042	0.019	0.104	0.042	0.020
	Playb. x time				-0.032	0.044	0.472
	Playb.x act _{meas}				0.035	0.030	0.256
T_a=27°C	Intercept	0.346	0.033	< 0.001	0.338	0.032	< 0.001
	Playback	-0.055	0.021	0.016	-0.038	0.025	0.138
	Mass _{init}	-0.003	0.010	0.777	-0.001	0.010	0.941
	Time	-0.018	0.013	0.169	<-0.001	0.016	0.989
	Trial	-0.036	0.017	0.035	-0.39	0.016	0.020
	Activity _{meas}	-0.002	0.010	0.803	0.005	0.011	0.663
	Sex	0.037	0.021	0.093	0.040	0.021	0.075
	Playb. x time				-0.037	0.024	0.135
	Playb.x act _{meas}				-0.025	0.016	0.139

Table S8: Outputs of the reduced and full linear mixed-effects models on evaporative cooling efficiency (EHL/MHP) of calm birds above the inflection point* (n=217 observations for 34 birds), at the maximal T_a reached (i.e. T_a=42°C or 44°C, n=32 observations for 19 birds), within the thermoneutral zone (T_a=35°C, n=67 for 34 birds) and at mild temperature (T_a=27°C, n=677 for 34 birds). The reference is the control group for playback, morning for time-of-day (here “Time”), trial 1 for trial, and female for sex. Est. ± SE corresponds to estimate ± standard error. Bold font shows significant effect (p<0.05). * Evaporative cooling efficiency (EHL/MHP) did not show any inflection point as a function of T_a from 35°C (Davies test, p=1).

- 1 Mariette, M. M. & Buchanan, K. L. Prenatal acoustic communication programs offspring for high posthatching temperatures in a songbird. *Science* **353**, 812-814 (2016).
- 2 Mariette, M. M., Cathaud, C., Chambon, R. & Vignal, C. Juvenile social experience affects pairing success at adulthood: congruence with the loser effect? *Proc. Royal Soc.* **280**, 20131514 (2013).
- 3 Lighton, J. R. *Measuring metabolic rates: a manual for scientists*. (Oxford University Press, 2008).
- 4 Withers, P. C., Cooper, C. E., Maloney, S. K., Bozinovic, F. & Cruz-Neto, A. P. *Ecological and environmental physiology of mammals*. Vol. 5 (Oxford University Press, 2016).
- 5 Tracy, C.R., Welch, W.R., Pinshow, B. & Porter, W.P. Properties of air: a manual for use in biophysical ecology. 4th Ed. *The University of Wisconsin Laboratory for Biophysical Ecology: Technical Report* (2010)