

Electronic Supplementary Materials

Early-life environmental effects on mitochondrial aerobic metabolism: a brood size manipulation in wild great tits

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A. Parental feeding rates

a) Material and Methods

In order to test if parental feeding rates changed following the brood size manipulation, we video-recorded a subsample of nest boxes ($n_C = 8$, $n_E = 15$, $n_R = 14$ nest boxes) 8 days after hatching. The cameras were concealed at ca. 2 m distance from the nest boxes. Videos were recorded for approximately 2h (mean \pm SD = 137.58 \pm 25.19 min) between 7 and 12 am. Standardized parental feeding rate differences (number of nest visits divided by the total length of the video starting from the first visit) was quantified using *BORIS* software (Friard & Gamba, 2016), by a single observer blind to the experimental treatment.

Standardized parental feeding rate differences (i.e. total number of visits per hour in the nest by both parents) were tested according to treatment groups and the initial brood size, but also according to the number of nestlings at day 7, using in both cases a linear model without random effects (LM). We included the starting time of the video recordings as a covariate in models to account for differences in feeding rates during the day.

b) Results

Parental feeding rate (8 days after hatching) was significantly affected by the treatment ($F_{2,32} = 4.64$, $P = 0.02$, see Fig.2A) with higher rates for the E group (raw data mean \pm SE = 41.26 \pm 6.03 visits per hour) compared to R group (raw data mean \pm SE = 25.75 \pm 4.05) (Tukey HSD *post hoc* comparison: $P = 0.04$). Differences in parental feeding rate between E and C groups (C: raw data mean \pm SE = 28.49 \pm 5.22) were close to significance (Tukey HSD *post hoc* comparison: $P = 0.051$). Parental feeding rate significantly increased with initial brood size (estimate \pm SE = 2.76 \pm 1.55, $F_{1,32} = 7.91$, $P = 0.008$) and significantly decreased with time of day (estimate \pm SE = -2.67 \pm 6.13e-10, $F_{1,32} = 19.01$, $P < 0.001$).

Parental feeding rate significantly increased with the number of nestlings recorded 7 days after hatching (estimate \pm SE = 4.28 \pm 1.01, $F_{1,34} = 22.41$, $P < 0.001$).

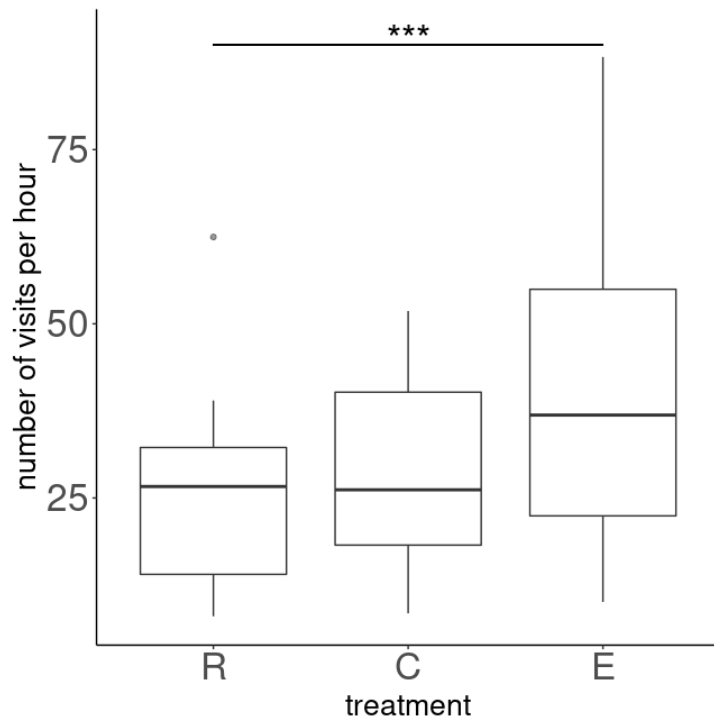


Fig. S1: Parental feeding rate according to the brood size manipulation treatment groups: reduced (R), control (C), enlarged (E) brood sizes. Raw data distribution is presented with boxplots ($n_C = 8$, $n_E = 15$, $n_R = 14$ nest boxes). Stars indicate the significance of Tukey HSD *post hoc* test (***) $P < 0.001$. $R^2 = 0.53$.

B) Results for the correlative approach

Table S1: Results of linear mixed model testing the associations between the number of nestlings in the nest and A) nestling body mass at day 7, B) nestling body mass at day 14, C) nestling wing length at day 14. For A), nestling body mass measured at day 2 was included as covariate in the model. For B), nestling body mass measured at day 7 was included as covariate in the model. Linear mixed models (LMM) estimates are reported with their 95% CI. Original nest box ID and nest box of rearing ID were included as random intercepts in the models. σ^2 , within group variance; τ_{00} between-group variance. Bold indicates significance ($P < 0.05$).

	A) Mass day 7			B) Mass day 14		
Predictors	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value
(Intercept)	2.15	-1.34 - 5.65	0.223	4.42	0.32 - 8.51	0.035
previous mass measured (day 2 or day 7)	1.91	1.75 - 2.06	<0.001	0.55	0.49 - 0.62	<0.001
number of nestlings	-0.16	-0.29 - -0.03	0.017	0.03	-0.13 - 0.18	0.726
hatching date	0.07	0.01 - 0.12	0.027	0.11	0.04 - 0.18	0.003
Random effects						
σ^2	0.60			0.60		
τ_{00} nest of origin	0.27			0.14		
τ_{00} nest of rearing	1.07			1.75		
N observations	419			403		
Marginal R^2 / Conditional R^2	0.525/ 0.852			0.348/ 0.844		

	C) Wing length day 14		
Predictors	Estimates	CI 95%	P-value
(Intercept)	20.79	13.77 - 27.81	<0.001
number of nestlings	0.23	-0.003 - 0.46	0.053
hatching date	0.42	0.30 - 0.54	<0.001
Random effects			
σ^2	5.60		
τ_{00} nest of origin	2.68		
τ_{00} nest of rearing	2.30		
N observations	403		
Marginal R^2 / Conditional R^2	0.345/ 0.653		

Table S2: Results of linear mixed models testing the associations between the number of nestlings in the nest (14 days after hatching) and mitochondrial respiration rates measured on 14-day-old nestlings (N = 102 individuals, n = 55 nest boxes). Mitochondrial respiration rates were corrected for the mitochondrial DNA copy number (i.e., proxy of mitochondrial density). Linear mixed models (LMM) estimates are reported with their 95% CI. Original nest box ID and nest box of rearing ID were included as random intercepts in the models. σ^2 , within group variance; τ_{00} between-group variance. Bold indicates significance ($P < 0.05$).

	ROUTINE			CI			CI + II			LEAK		
Predictors	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value
(Intercept)	4.55	2.37 – 6.72	<0.001	20.12	12.93 - 27.31	< 0.001	29.39	18.21 – 40.57	<0.001	2.70	1.20 – 4.20	<0.001
number of nestlings	-0.13	-0.22 – -0.04	0.005	-0.44	-0.72 – -0.17	0.002	-0.66	-1.09 – -0.23	0.003	-0.10	-0.16 – -0.04	<0.001
mtDNA cn	0.34	0.25 – 0.42	<0.001	0.91	0.69 – 1.12	<0.001	1.44	1.10 – 1.77	<0.001	0.18	0.14 – 0.23	<0.001
hatching date	-0.02	-0.06 – 0.02	0.305	-0.17	-0.29 – -0.04	0.009	-0.24	-0.43 – -0.05	0.013	-0.01	-0.04 – 0.01	0.384
Random effects												
σ^2	0.32			1.31			3.13			0.06		
τ_{00} nest of origin	0.05			1.10			2.52			0.04		
τ_{00} nest of rearing	0.33			4.21			10.35			0.19		
Observations	102			102			102			102		
Marginal R^2 / Conditional R^2	0.488 / 0.767			0.487 / 0.898			0.483 / 0.899			0.454 / 0.889		

C. Complementary analyses, results for the control group only

Table S3: Results of linear mixed models testing the associations between the number of nestlings in the nest (14 days after hatching) and mitochondrial respiration rates measured on 14-day-old nestlings (N = 26 individuals from the control group only). We found similar results as in statistical analyses conducted on the whole data set (same direction for significant effects). Mitochondrial respiration rates were corrected for the mitochondrial DNA copy number (i.e., proxy of mitochondrial density). Linear mixed models (LMM) estimates are reported with their 95% CI. The original nest box ID and the nest box of rearing ID were both included as random intercepts in the models. σ^2 , within group variance; τ_{00} between-group variance. Bold indicates significance ($P < 0.05$).

	ROUTINE			CI			CI + II			LEAK		
Predictors	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value
(Intercept)	6.85	1.13 – 12.57	0.026	27.36	12.74 – 41.98	0.002	42.52	18.77 – 66.28	0.002	4.84	2.21 – 7.48	0.002
number of nestlings	-0.26	-0.65 – 0.12	0.162	-1.18	-2.14 – -0.22	0.020	-1.82	-3.38 – -0.25	0.026	-0.25	-0.42 – -0.09	0.007
mtDNAcn	0.29	-0.03 – 0.60	0.070	0.54	-0.26 – 1.34	0.175	0.88	-0.39 – 2.16	0.163	0.14	0.01 – 0.26	0.037
hatching date	- 0.04	-0.15 – 0.07	0.433	-0.18	-0.46 – 0.10	0.179	-0.30	-0.75 – 0.16	0.181	- 0.03	-0.08 – 0.02	0.252
Random effects												
σ^2	0.53			2.95			6.58			0.05		
τ_{00} nest of origin	0.01			1.47			3.28			0.08		
τ_{00} nest of rearing	0.45			2.56			8.35			0.09		
Observations	26			26			26			26		
Marginal R ² / Conditional R ²	0.483 / 0.724			0.585 / 0.824			0.571 / 0.845			0.684 / 0.932		

D. Complementary analyses, results without including the small brood sizes

Table S4. Results of linear mixed models testing the associations between the number of nestlings in the nest (14 days after hatching) and mitochondrial respiration rates measured on 14-day-old nestlings (N = 90 individuals from 46 nests, broods having less than 5 nestlings at day 14 are not included in the analyses). Mitochondrial respiration rates were corrected for the mitochondrial DNA copy number (i.e. proxy of mitochondrial density). Linear mixed models (LMM) estimates are reported with their 95% CI. The nest box of rearing ID was included as random intercept in the models. Nest box of origin could not be included as random intercept because of convergence issues. σ^2 , within group variance; τ_{00} between-group variance. Bold indicates significance ($P < 0.05$).

	ROUTINE			CI			CI + II			LEAK		
Predictors	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value	Estimates	CI 95%	P-value
(Intercept)	3.84	1.53 – 6.16	0.002	16.69	9.32 – 24.07	<0.001	24.27	12.82 – 35.72	<0.001	2.37	1.06 – 3.69	0.001
number of nestlings	-0.09	-0.20 – 0.03	0.143	-0.29	-0.66 – 0.08	0.117	-0.39	-0.96 – 0.19	0.182	-0.04	-0.10 – 0.03	0.247
mtDNAcn	0.30	0.20 – 0.40	<0.001	0.74	0.48 – 1.00	<0.001	1.18	0.78 – 1.59	<0.001	0.15	0.10 – 0.20	<0.001
hatching date	-0.01	-0.05 – 0.03	0.563	-0.12	-0.24 – -0.002	0.055	-0.18	-0.36 – 0.01	0.062	-0.01	-0.03 – 0.01	0.246
Random effects												
σ^2	0.33			2.09			4.91			0.08		
τ_{00} nest of rearing	0.32			3.91			9.51			0.12		
Observations	90			90			90			90		
Marginal R ² / Conditional R ²	0.293 / 0.643			0.250 / 0.739			0.248 / 0.744			0.248 / 0.682		

E. Results for the association between mitochondrial respiration rates in 14-days-old nestlings and survival as juvenile

Table S5. Results of generalized linear mixed models (GLMM ,with logistic binary distributions of the dependent variables, survival: 0=dead, 1=alive) testing whether mitochondrial respiration rates measured at day 14 predict juvenile recapture probability (i.e. proxy of medium-term apparent survival). Models only include individuals for which mitochondrial metabolic rates have been measured at day 14 (N = 102 individuals). 67 individuals (from 34 nests) have been recaptured as juveniles. Random intercepts could not be included in the models because of convergence issues. Odds ratios are reported with their 95% CI. R² values are estimated from the *coefficient of determination D* (Tjur's approach). Bold indicates significance ($P < 0.05$).

Predictors	ROUTINE			CI			CI+II			LEAK		
	Odds Ratios	CI 95%	P-value	Odds Ratios	CI 95%	P-value	Odds Ratios	CI 95%	P-value	Odds Ratios	CI 95%	P-value
(Intercept)	82.24	0.43 – 1.7e4	0.10	60.96	0.26 – 1.4e4	0.14	75.12	0.35 – 1.7e4	0.11	114.31	0.67 – 2.2e4	0.07
Mitochondrial respiration rate d14	1.30	0.87 – 1.95	0.20	1.08	0.95 – 1.23	0.24	1.04	0.96 – 1.14	0.31	1.33	0.66 – 2.47	0.38
hatching date	0.89	0.80 – 0.97	0.01	0.89	0.81 – 0.98	0.01	0.89	0.80 – 0.97	0.01	0.89	0.80 – 0.98	0.01
Observations	102			102			102			102		
R ²	0.09			0.09			0.08			0.08		

References:

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open- source event- logging software for video/audio coding and live observations. *Methods in ecology and evolution*, 7(11), 1325-1330.