Helga Gwinner, Pablo Capilla-Lasheras, Caren Cooper & Barbara Helm:

"Green incubation": avian offspring benefit from aromatic nest herbs through improved parental incubation behaviour

Supplementary Methods

Derivation of on-bouts and off-bouts

We used consecutive logger readings of egg temperature to derive times when parents were likely to be present (i.e., on-bouts) or absent (i.e., off-bouts) from their nests (for details, see Capilla-Lasheras 2018). Supplementary Methods Fig. 1 shows an exemplary 24 h trace of egg temperatures recorded from a nest-box. We inferred the beginning of off-bouts by identifying temperature drops between consecutive logger readings that exceeded the differences measured during a reference period when parents were most likely to sit tightly on their nests. As reference period for extracting each individual's daily thresholds, we used the core time of the preceding night, defined to last from 22:00 until 3:30 (CET, summer savings time) on the following morning. We then compared consecutive logger readings during daytime (from 3:30 to 22:00) to this threshold and marked the times when it was crossed as the beginning of an off-bout. Off-bout periods were defined as ending when temperatures increased again, indicating that the parent had returned to the nest. The result of this algorithm was a temporal sequence of 1's (on-bouts; Supplementary Methods Fig. 1, red) and 0's (off-bouts; blue), respectively. The algorithm was implemented using customised R scripts (Capilla-Lasheras 2017).

Supplementary Methods Figure 1. 24 h trace of egg temperatures recorded from a European starling. Data are temperature recordings from iButtons plotted against time; dots show individual readings and their assignment as either on-bouts (red) or off-bouts (blue).
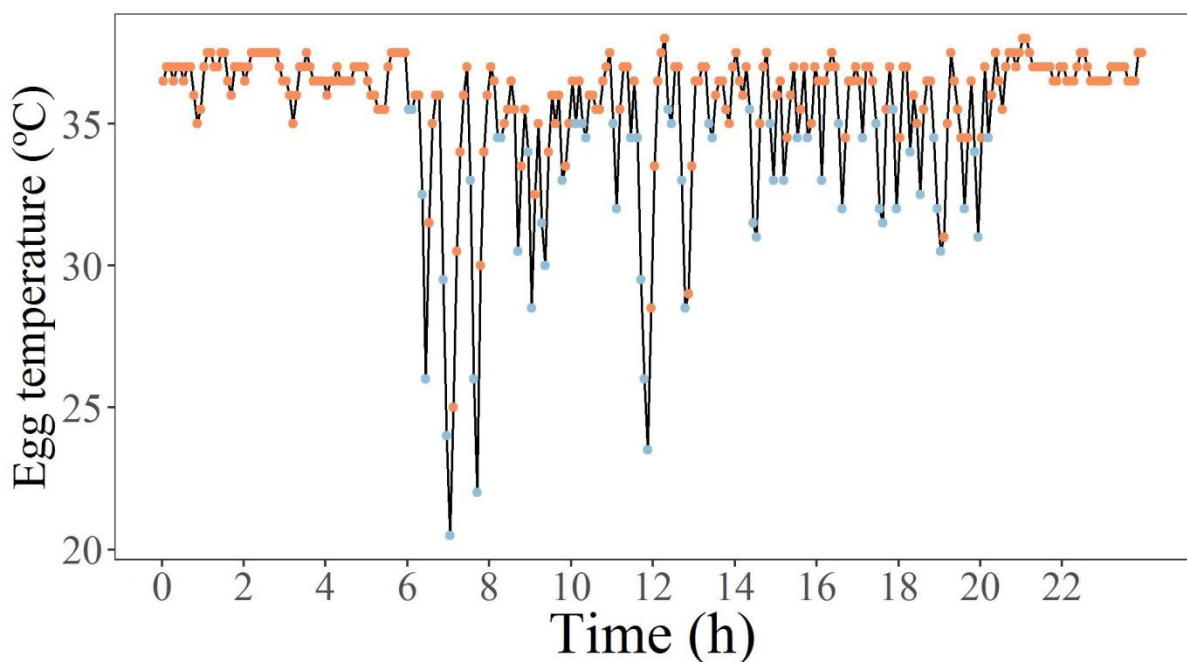
Delineation of day and night

For several analyses we separated the data into day and night, using either astronomical or behavioural delineations. Astronomical delineation used civil twilight, such that "day" was defined as the interval between the rising of the sun above -6° relative to the horizon at dawn and its sinking below this angle at dusk, based on civil twilight times from the US Naval Observatory (http://aa.usno.navy.mil/data/), and referring to local time (CET, summer savings time). This delineation was used to calculate mean and variance of egg temperatures per nest-box for each day and night of incubation.

In contrast, when analysing inferred incubation behaviour of the parents, we derived day and night from the birds' recorded activities. We defined daytime behaviourally as the time between the start of a bird's first off-bout in the morning (first departure) until the end of its last off-bout in the evening (last return) relative to civil twilight. Morning activity onset was calculated as the time of first morning civil twilight minus the time of first departure. Hence, if a bird first left the nest-box before civil twilight started it had a positive value for activity onset for that day, and if it first left after civil twilight had started it had a negative value. In the evening, offset of activity was calculated as the time of last evening civil twilight minus the time of last return. Hence, if a bird last returned to the nest-box before civil twilight ended it had a positive value for activity offset for that day, and if it last returned after civil twilight ended it had a negative value.

Exclusion of data

Of the overall data-set, we used 184 observation days from herb nests and 160 from herbless nests in the final analysis, employing exclusion criteria. For the analysis of daytime mean temperature, temperature variation and drops below 32°C, we excluded days with data gaps (i.e. days around mid-incubation when data were downloaded). Similarly, for night-time temperature mean and variation, we excluded 6 nights with long data gaps, and 2 nights with extended deep temperature drops (lasting 1.8 and 2.5 h), when the parent was evidently absent and possibly disturbed. When analysing onset and offset of daily activity, data points within 2 h before and after morning and evening civil twilight, respectively, were used. If no changes were recorded within this time window, no onsets or offsets were specified. For calculating attendance and number and duration of off-bouts, days with data gaps, or with onset / offset of activity excluded, were not used in the analyses.

Controlled cooling experiment

In the main manuscript, we investigated potential differences in thermal properties of the nest-types by comparing cooling during the parents' extended midday outings. In addition, we also tested cooling differences in isolation from birds in an incubator in the laboratory. We created artificial nests, exactly like those used in the experiment, which either did (fresh herb, n = 8), or did not (herbless, n = 3), contain fresh green herbs.We also created herb nests that contained the same plant composition but dried (dry herb, n = 2), to separate effects of herbs from those of fresh vs. dry material. In each nest we inserted a logger egg, and then placed it in an incubator that was preheated to 35°C. After leaving the nest and logger egg in the incubator to reach the preset temperature, we turned off the incubator and recorded egg temperature drops from 35°C to 25°C. As evident from Supplementary Methods Fig. 2, there was no indication of any differences between the nest-types in cooling curves. This further supported our conclusion that the differences we report for egg-temperatures of wild starlings were caused by the birds' behaviour, and not by thermal properties of the different nest types.

Supplementary Methods Figure 2. Cooling curves of egg temperatures recorded in an incubator in three types of artificial starling nests. Data are temperature recordings from iButtons in logger-eggs, plotted against time; dots show individual readings.
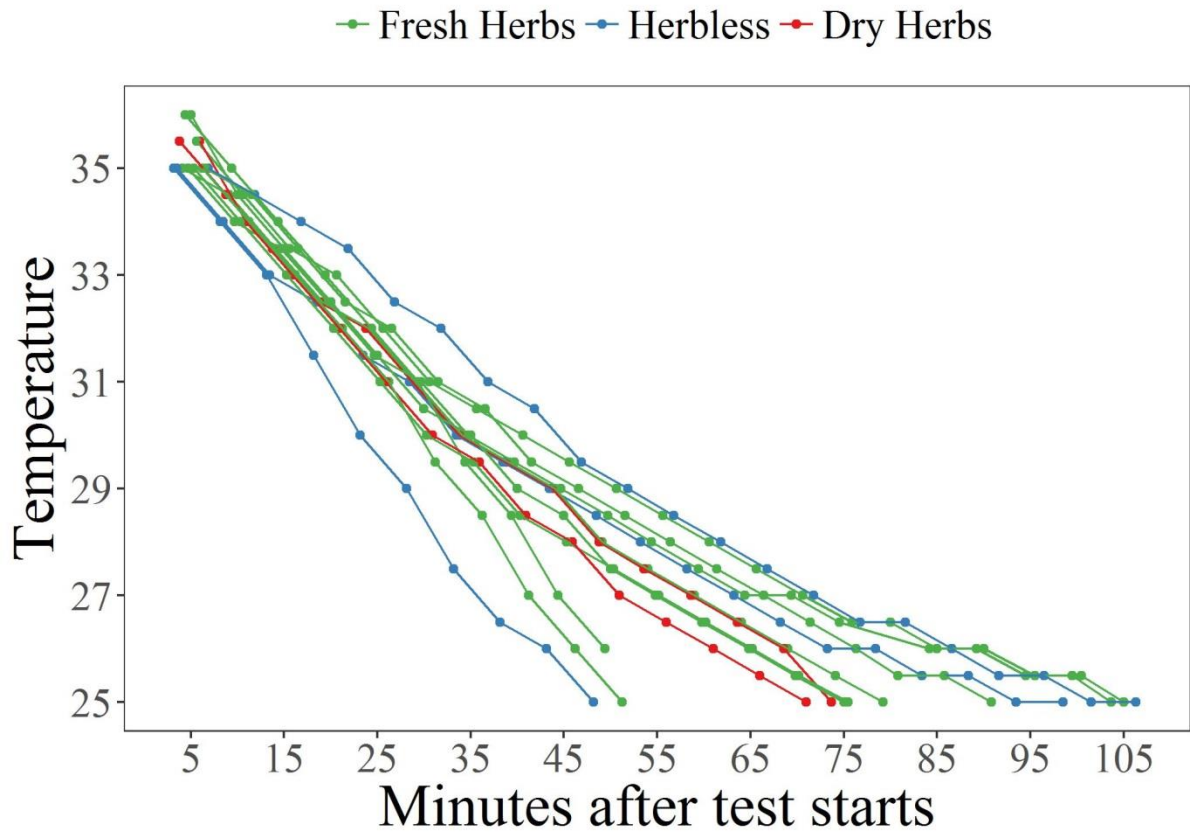
References

Capilla-Lasheras, P. 2018. incR: a new R package to analyse incubation behaviour. *Journal of Avian Biology, in press*.

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Supplementary Table 1. Statistical assessment of predictors explaining variation in egg temperature, inferred parental behaviour and breeding parameters in herb and herbless nests of starlings. Model selection using likelihood-ratio tests (LRT). Shown are results for fully nested LRTs, starting from the most complex models and sequentially dropping non-significant terms. When higher-order terms were retained in the model, lower-order terms could not be dropped separately from the model. For estimates, and for details on the contributions of lower-order terms to significant higher-order effects, see summary of the minimal adequate models in Table 1. A) Daytime and night-time temperatures of eggs, analyzed for daily means and variances per nest across the incubation period. B) Thermal characteristics of the nest-types derived from the birds' extended midday outings; analyzed are temperature upon return, cooling rate, and time taken to re-warm eggs to 35º C (h). C) Parental behaviour, inferred from changes between consecutive temperature readings; analyzed are attendance (proportion of time on nest over 24 h), daytime numbers of static phases on the nest per day, duration of egg temperature < 32º C (h), number and duration (min) of off-bouts per day, and timing of first morning departure (h before morning civil twilight) and last evening return (h before evening civil twilight). D) Offspring development and breeding success, analyzed for hatching success, developmental score per nest on incubation day 13, and nestling body mass 1 week after hatching controls. Year and Nest-box within Year were always included as random factors. "Day" refers to incubation day; [a]logarithmically transformed; [b]Poisson model; [c]binominal model.

A) Effects of herbs on egg temperatures

Response variable	Predictor	χ	df	p-value
Mean daytime egg temperature	Day^{2}x Nest-type	3.34	1	0.068
	Day^{1}x Nest-type	4.43	1	0.035
	Day^2	167.3	1	< 0.001
	Day^{2}x Nest-type	1.2	1	0.281
	Day^{1}x Nest-type	0.069	1	0.793
Variance daytime egg temperature[a]	Day^2	64.11	1	<0.001
	Nest-type	5.2	1	0.023
Mean night-time egg temperature	Day^{2}x Nest-type	0.19	1	0.662
	Day^{1}x Nest-type	5.51	1	0.019
	Day^2	100.94	1	<0.001
	Day^{2}x Nest-type	0.31	1	0.58
	Day^{1}x Nest-type	1.22	1	0.27
Variance night-time egg temperature[a]	Day^2	8.77	1	0.003
	Nest-type	1.2	1	0.273

B) Effects of herbs on nest thermal properties during midday outings

Response variable	Predictor	χ	df	p-value
	Day^2 x Nest-type	0.18	1	0.67
	Day^1 x Nest-type	0.23	1	0.63
	Day^2	0.72	1	0.40
Temperature upon return	Day	3.98	1	0.046
	Nest-type	0.01	1	0.917
	Duration of outing	53.68	1	<0.001
	Temperature at start	47.01	1	<0.001
	Day^2 x Nest-type	1.03	1	0.308
	Day^1 x Nest-type	0.43	1	0.513
Cooling rate[a]	Day^2	2.27	1	0.131
	Day	2.00	1	0.157
	Nest-type	2.02	1	0.156
	Day^2 x Nest-type	1.62	1	0.203
	Day^1 x Nest-type	0.26	1	0.609
	Day^2	0.00	1	0.995
Time to rewarm (h)	Day	17.75	1	<0.0001
	Nest-type	2.13	1	0.145
	Temperature upon return	48.61	1	<0.001

C) Effects of herbs on inferred incubation behaviour

Response variable	Predictor	χ	df	p-value
Attendance	Day^2 x Nest-type	0.92	1	0.339
	Day^1 x Nest-type	6.72	1	0.009
	Day^2	21.97	1	<0.001
	Day^2 x Nest-type	0.08	1	0.778
	Day^1 x Nest-type	3.68	1	0.055
Number of daytime static phases[b]	Day^2	23.42	1	<0.001
	Nest-type	2.68	1	0.102
Duration of drops < 32ºC (h)[a]	Day^2 x Nest-type	6.01	1	0.014
Number of daytime off-bouts[b]	Day^2 x Nest-type	0.28	1	0.595
	Day^1 x Nest-type	7.43	1	0.006
	Day^2	0.11	1	0.738
Duration of daytime off-bouts (min)	Day^2 x Nest-type	4.07	1	0.044
First morning departure (h)	Day^2 x Nest-type	6.43	1	0.011
Last evening return (h)	Day^2 x Nest-type	0.09	1	0.759
	Day^1 x Nest-type	3.06	1	0.080
	Day^2	1.85	1	0.174
	Day	12.91	1	<0.001
	Nest-type	0.61	1	0.436

D) Effects of herbs on hatching success and offspring development

Response variable	Predictor	χ	df	p-value
Hatching success[c]	Nest-type	0.47	1	0.494
Developmental score	Nest-type	7.34	1	0.007
Nestling weight (all chicks)	Inferred nestling agex Nest-type	0.56	1	0.455
	Inferred nestling age	1.73	1	0.189
	Nest-type	7.21	1	0.007
Nestling weight (low-mass chicks removed)	Inferred nestling agex Nest-type	0.95	1	0.330
	Inferred nestling age	3.51	1	0.061
	Nest-type	8.02	1	0.005

Supplementary Table 2. Variation in nestling mass in herb and herbless nests after removal of four low-body mass chicks. Estimated coefficients and test statistics for the minimal adequate models selected using likelihood-ratio tests. Linear mixed models (LMM) were used to estimate coefficients and test statistics for all terms contained in the final models. Nest-type was always included in the final model. Model output shows coefficients, standard errors (SE), degrees of freedom (df), t-values (Z scores) and p-values. Year and Nest-box ID within Year were included as random factors. Nest-type (herbs) was set as reference level; accordingly, the coefficient for Nest-type (herbless) represents the difference between the two nest-types.

| Nestling weight					
Predictor	**Coefficient**	**SE**	**df**	**t-value**	**p-value**
Intercept	15.47	18.75	52	0.83	0.413
Inferred nestling age	4.67	2.57	21	1.82	0.084
Nest-type	-6.18	2.14	21	-2.88	0.009

Supplementary Movie and Figures

Supplementary Movie. Movie of a male starling in our study colony performing courtship display in front of his nest-box, showcasing green plant material. Credit: Jens Uwe Heins.

Supplementary Figure 1. Picture of a natural starling nest in our study colony containing herbs provisioned by the male. Picture: Helga Gwinner.

Supplementary Figure 2. Inferred duration of the parents' extended mid-day outings (min). Data are plotted over the course of incubation separately for herb and herbless nests; shown are raw data points (small dots) and model predictions ±SE (large dots) based on minimal adequate models (Table 1). Points are slightly offset within days to aid visibility.
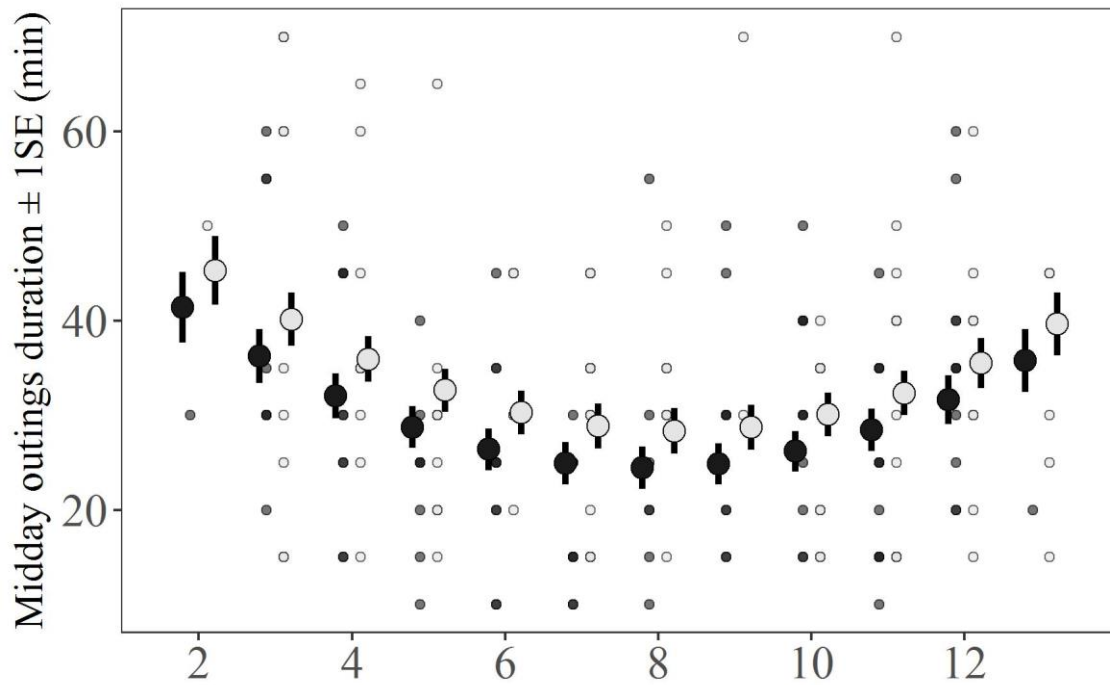
Supplementary Figure 3. Inferred number of phases when parents sat statically on the nest. Data are plotted over the course of incubation separately for herb and herbless nests; shown are raw data points (small dots) and model predictions ±SE (large dots) based on minimal adequate models (Table 1). Points are slightly offset within days to aid visibility.
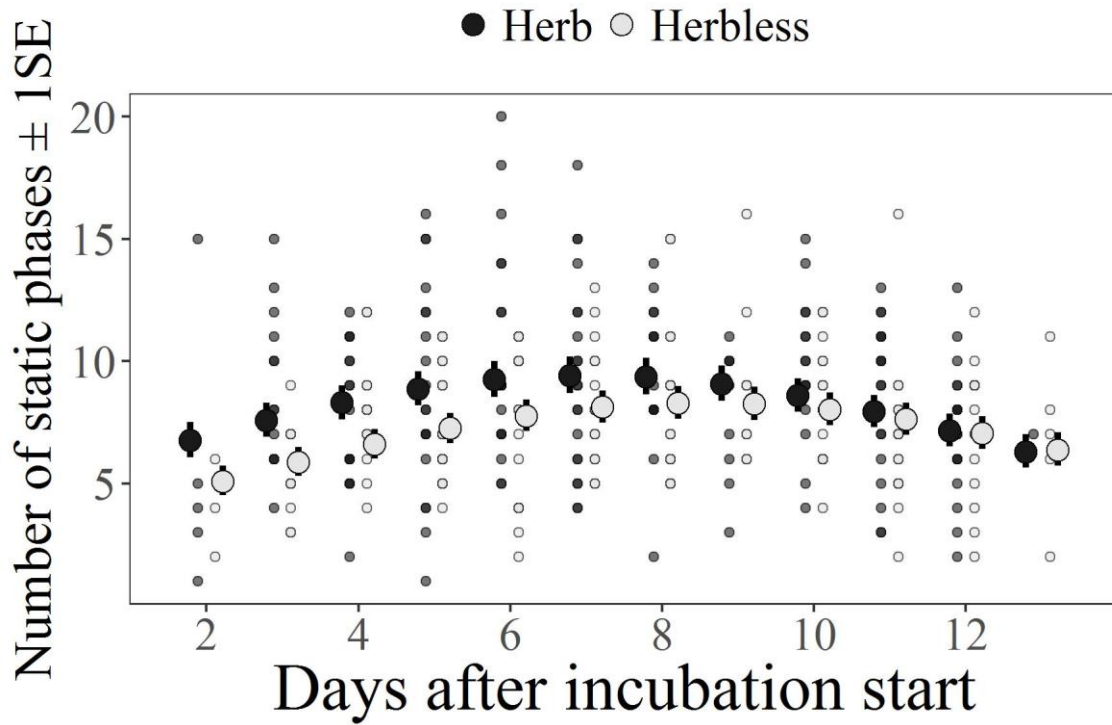
Supplementary Figure 4. Inferred duration of daytime egg temperature drops <27°C (h). Data are plotted over the course of incubation separately for herb and herbless nests; shown are raw data points (small dots) and model predictions ±SE (large dots) based on minimal adequate models (Table 1). Points are slightly offset within days to aid visibility.
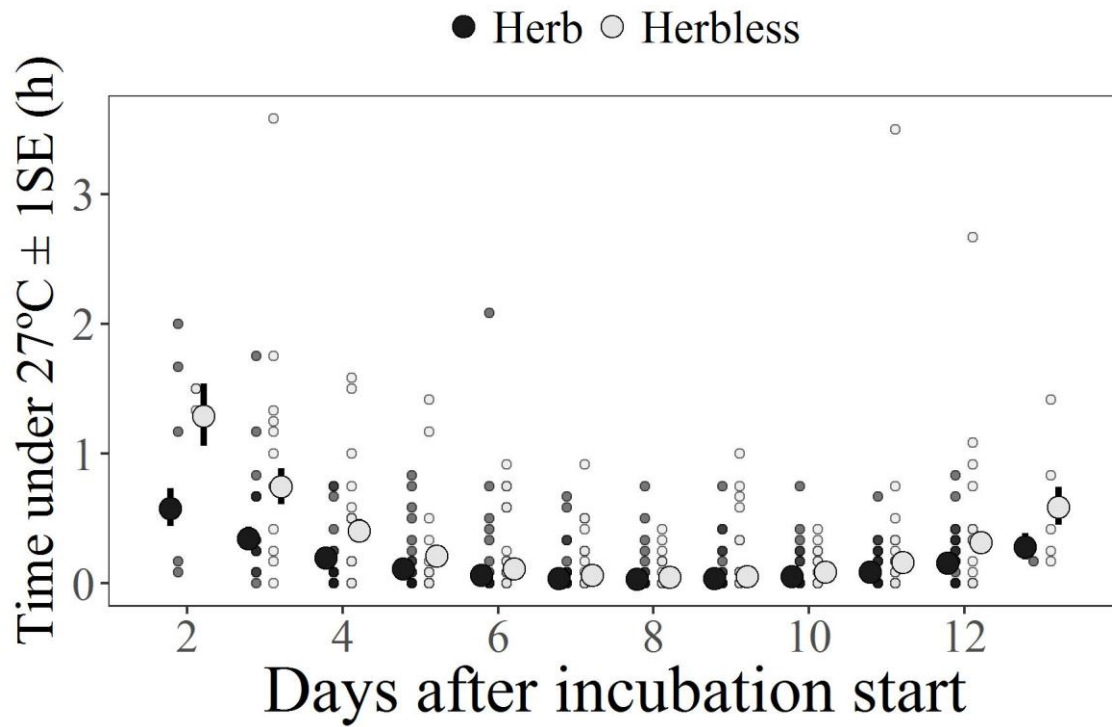
Supplementary Figure 5. Timing of the starling's active day relative to morning onset and evening end of civil twilight. Data are plotted over the course of incubation separately for herb and herbless nests. Top panel: last evening return to nest-box, bottom panel: first morning departure; details as in Fig. 1.
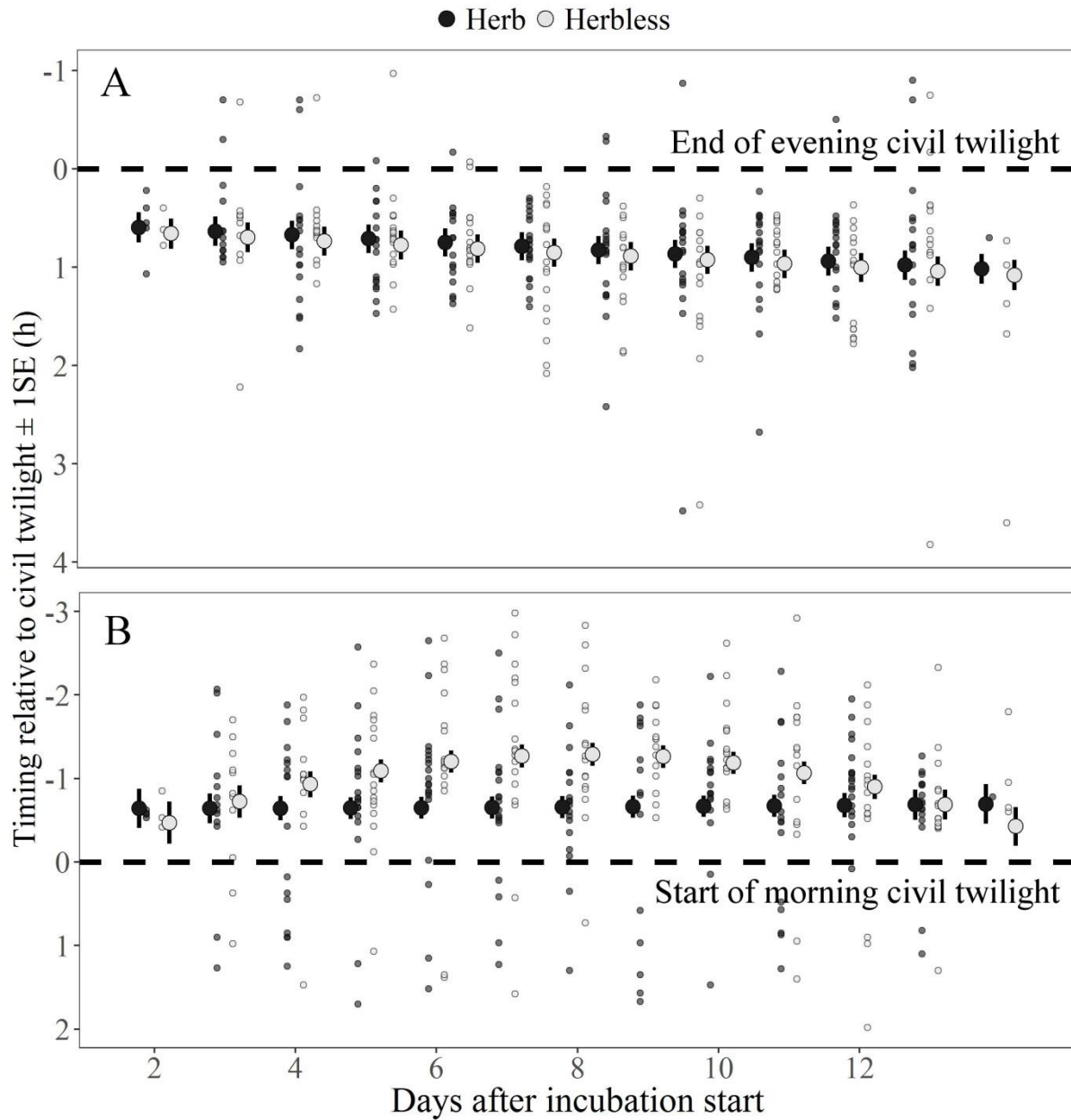
Supplementary Figure 6. Nestling body mass in herb and herbless nests depending on age, excluding four nestlings with very low body mass. Shown is body mass at mean clutch ages from 6.5 to 8 days; details as in Fig. 4.