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Temperature change is an important departure cue in nocturnal migrants: controlled experiments with wild-caught birds in a proof-of-concept study

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The decision-making process of migrating birds at stopover sites is a complex interplay of the innate migration program and both intrinsic and extrinsic factors. While it is well studied how variation in precipitation, wind and air pressure influence this process, there is less evidence of the effects of temperature changes on the departure decision. Thus, we lack knowledge on how the predicted changes due to global climate change in temperature alone may affect the decision-making process during migration. Aiming to fill parts of this gap, we conducted a proof-of-concept study by manipulating the ambient temperature of temporarily confined wild-caught migrant songbirds under constant feeding conditions. In spring, departure probability increased with a 20°C rise in temperature for both a medium-distance migrant (European robin, *Erithacus rubecula*) and a long-distance migrant (northern wheatear, *Oenanthe oenanthe*), and in autumn, departure probabilities of the long-distance migrant both decreased with a 20°C rise and increased with a 20°C drop. Consequently, the temperature is an important departure cue influencing the decision-making process of migrating songbirds. Incorporating causal relationships between changes in temperature and departure probability in migration models could substantially improve our ability to predict the effects of climate change on the phenology of migratory birds.

1. Background

Migrating birds often travel between their breeding areas and wintering grounds using a stop-and-go strategy [1]. They stop over at suitable sites to rest [2], recover [3] and/or feed to accumulate energy for the subsequent flight(s) [4]. The decision-making process of when to resume migration from a stopover site is the result of the highly complex interplay of the innate migration program (e.g. circannual and circadian rhythms), intrinsic factors (e.g. energy stores and physiological conditions) and extrinsic factors (e.g. feeding conditions and meteorological conditions) [4–6].

A large number of studies have investigated the influence of meteorological conditions on the departure decision in birds, e.g. wind, precipitation and air pressure [6]. However, we know relatively little about the effects of air temperature on the decision-making process. So far, there is correlative evidence from free-flying migrants that departure probability increases with a temperature rise, as shown in pink-footed geese (*Anser brachyrhynchus*) [7] and greater white-fronted geese (*Anser albifrons albifrons*) [8] during spring migration, and with a temperature drop, as shown in greater white-fronted geese (*Anser albifrons*) during autumn migration [9]. The latter pattern was also found in the northern wheatear (*Oenanthe oenanthe*) [10,11]; though this reaction to a temperature change was flexible in space and time [12].

Despite the relevance of studying migration behaviour under natural conditions, these correlative studies could not distinguish whether the departure decision was affected by temperature alone and/or food availability. For instance, the costs of thermoregulation are increased with a temperature drop [13,14]. To counterbalance the corresponding higher energy demand, a bird would need to forage more that in turn substantially increases its metabolic rate. Since food availability is (at least to a certain extent) temperature-dependent with lower access to plants and arthropods under snowy and/or colder conditions [15,16], we need to break this dependency in order to test for a causal relationship between changes in temperature and departure decisions of migrants. This requires to experimentally manipulate the temperature conditions while keeping all other conditions, such as feeding conditions, constant. Such laboratory experiments would provide essential but currently still lacking knowledge on how the predicted changes in temperature alone may affect the decision-making process of migrants.

The first step in this direction was carried out by Berchtold *et al.* [17] conducting an indoor experiment with white-crowned sparrows (*Zonotrichia albicollis*), a short-distance migrant, during autumn. Individual caged birds were exposed to cool (4°C), medium (14°C) and warm temperatures (24°C) and each night assessed with respect to their migratory restlessness. Caged songbirds show this behaviour at times of the year and day when they would normally migrate in the wild [18] and its amount is a good approximation for the individual departure probability from a stopover [19]. Because the white-crowned sparrows showed little migratory activity at cool and medium temperatures, but none at the warm temperature, Berchtold *et al.* [17] argued that temperature is important in the decision-making process during migration. As far as we know, this is the only experiment investigating the effects of temperature change on departure probability. Therefore, we lack experimental assessment of how variation in temperature may affect the departure probability in spring and whether medium- and long-distance migrant birds may react differently to a temperature change. The latter is potentially important because short-, medium- and long-distance migrants may react differently to variation in environmental conditions [20,21]. For instance, wind conditions were most important to explain variation in the autumn phenology of medium-distance migrants, whereas temperature and precipitation significantly affected the phenology of long-distance migrants [20].

In order to fill parts of these knowledge gaps, we temporally caged a medium-distance migrant, the European robin (*Erithacus rubecula*), wintering in central to southern Europe, and a long-distance migrant, the northern wheatear, wintering in sub-Saharan Africa (electronic supplementary material, figure S1), at a stopover site during spring and autumn migration. Both are nocturnal migrants [21]. To test the concept of an effect of temperature change on departure probability, the birds were initially exposed to either 5°C or 25°C, reflecting the natural variation in temperature at the site in spring and autumn (electronic supplementary material, figure S2), while other conditions were kept equal. The birds were provided with *ad libitum* food and water. During caging, birds were expected to initially accumulate energy stores and keep them at a high level [22]. This gain in energy stores results initially in higher nocturnal migratory restlessness, but this increase levels off after the first or

second day of feeding so that the amount of restlessness usually remains at about the same level afterwards [22,23]. In the morning of day 4, the birds first kept at 25°C were exposed to 5°C, and those first kept at 5°C to 25°C. We measured the birds' migratory restlessness as a proxy for their departure probability that night [19]. To study the immediate response of the birds to the temperature changes, we compared the migratory restlessness during the night before the temperature change with the amount during the night after the temperature change in a longitudinal approach.

For our proof-of-concept study, we predicted for spring that (i) a temperature rise, indicating improved temperature-related environmental conditions at the migratory destination (e.g. earlier snowmelt [24]), would increase the departure probability [25] to advance arrival at the breeding area [26], and (ii) a temperature drop, indicating increased energetic costs through higher metabolic rates [13], would increase the urge to move to a more suitable stopover site, although the *ad libitum* feeding conditions, representing a suitable site, might counterbalance the urge for such movements [4]. For autumn, we predicted that (iii) a temperature rise would reduce the departure probability because further exploitation of the *ad libitum* food sources would minimize the risk of starvation, mortality [27] and the time spent on migration [28], and (iv) a temperature drop would increase the departure probability, minimizing the energetic costs for migration [14] and probably avoiding unfavourable feeding and environmental conditions.

2. Methods

(a) Study site, species and trapping procedure

The study was conducted on Helgoland (54°11' N, 07°55' E), a small island roughly 50 km off the German North Sea coastline, in 2018. European robins (robins hereafter) and northern wheatears (wheatears hereafter) were captured during the species-specific main passage times (electronic supplementary material, figure S3). After trapping, wing length (maximum chord [29]) was measured to the nearest 0.5 mm, body mass to the nearest 0.1 g. All procedures were approved by the Ministry of Energy, Agriculture, the Environment, Nature and Digitalization, Schleswig-Holstein, Germany.

(b) Experimental design

The birds were placed into individual cages with a maximum of ten cages in each of two identical experimental indoor rooms (12 m²). Rooms were located at the Alfred-Wegener Institute for Polar and Marine Research on Helgoland. Low-pressure cooling compressors (Typ 2HC1.2 J-40S, Bitzer, Sindelfingen, Germany) regulated the temperature of the rooms, 25°C and 5°C. Birds were not stressed because they began feeding within several minutes of being placed in the cages and because caging did not result in indications of stress at least in wheatears [30]. Artificial lights were switched on/off at local sunrise/sunset. During the nights, dim white light conditions of about 2 lux (20 lamps, 8.0 V, 50 mA; Barthelme, #00640850, Nürnberg, Germany) were provided as in Maggini & Bairlein [31]. Birds had continual *ad libitum* access to water. *Ad libitum* food (i.e. 40 g of mealworms, *Tenebrio molitor*) was provided immediately after the daylight was switched on. Food trays were removed when the daylight was switched off and were never emptied by any bird allowing us to estimate the bird's daily

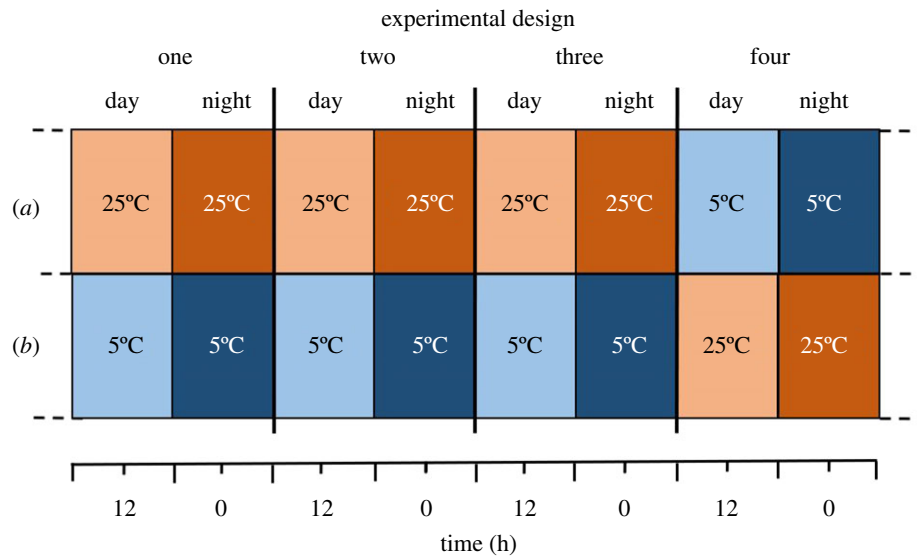


Figure 1. Experimental design. Darker colours indicate the nights during which nocturnal migratory restlessness was measured. Upon capture, birds were randomly divided into two groups: group A entered the room with an ambient temperature of 25°C (orange), group B entered the room kept at 5°C (blue). After the third night of captivity, group A was transferred to the room kept at 5°C, group B to the room kept at 25°C. Both groups received *ad libitum* food during the daylight period and *ad libitum* water throughout. Migratory restlessness was measured each night, but data of the first and second night were not considered for the main analyses because they were considered as an ‘acclimatization phase’ (for details see Methods and for data of all nights the electronic supplementary material, table S4 and figure S8). (Online version in colour.)

food intake. These estimates are not exact, however, because thrown-out or escaped mealworms could not be attributed to individual food trays. All birds were weighed daily to the nearest 0.1 g at local sunrise and sunset. Birds were randomly assigned to either the 25°C room or the 5°C room (figure 1). The day of catching and the second day, including experimental night two, were treated as the acclimatization period to the caging conditions and to allow the birds to gain energy stores. After the third night, each bird was transferred to the other room shortly after the daylights were switched on. Thus, the birds experienced a rapid increase (5°C to 25°C) or a decrease (25°C to 5°C) in ambient temperature (figure 1). After the experiment, all birds were released. We intended to study the response to a temperature change. Therefore, we assessed the change in the migratory restlessness between experimental nights three and four (figure 1). We did not keep control birds constantly at 5°C or 25°C because former experiments, also with *ad libitum* food, did not reveal an obvious change in the amount of migratory restlessness after the initial increase during the first, second or third day [22,23]. The changes in the amount of migratory restlessness after the third night are therefore not caused by the number of days in captivity or a change in the feed conditions [22], but they would be a response to a temperature change under the otherwise constant conditions.

(c) Recording of nocturnal migratory restlessness

We took advantage of the fact that caged songbirds show nocturnal migratory restlessness at times of the year and day when they would normally migrate in the wild [18] and that the amount of restlessness is predictive of the bird’s actual departure probability [19]. Quantifying migratory restlessness therefore allows a relative comparison of departure probability within and between individuals [19]. The restlessness behaviour includes wing whirring, flying, fluttering and hopping [32]. Since such movements generate an impulse through a change in air pressure, we attached motion-sensitive microphones (Piezo-Scheibe 27 mm, Conrad Electronics SE, Hirschau, Germany) to each cage (electronic supplementary material, figure S4). To avoid the recording of occasional non-migratory activity, we set a threshold of three impulses per second before

considering these as an activity count [31]. Activity counts were summed for 1 min periods over the entire night. We restricted the data to the first 10 h after sunset in spring and to the first 7 h after sunset in autumn, which corresponded to the shortest length of the night that the birds experienced in the respective season during this study. We excluded activity counts from the first 15 min of each night to avoid any potential effect of switching off the daylight. We used the remaining number of all 1 min activity periods per night as a proxy for the bird’s departure probability in that night [19].

(d) Energy stores

Fat, glycogen and protein serve as energy stores in birds. Since the contribution of energy derived from fat in relation to the total energy expenditure during flight is about 95% [33], we assessed the bird’s energy stores as its total amount of body fat. The evening energy stores were estimated as the bird’s specific lean body mass subtracted from its actual evening body mass divided by its lean body mass.

We used data from a quantitative magnetic resonance body composition analyser following Klinner *et al.* [22] to estimate lean body mass. This analyser provides precise and accurate direct measurements of, among others, the bird’s fat and lean mass in a non-invasive approach [34,35] and thus enables more robust estimates of energy stores than other biometric approaches or fat scoring. Variation in wing length of wheatears explained 66% of the variation in lean body mass according to the following linear regression:

$$\begin{aligned} \text{lean body mass (g)} &= 0.33 \text{ g mm}^{-1} \\ &\times \text{wing length mm} - 11.3 \text{ g}; \\ \text{adj. } R^2 &= 0.66, n = 37, p < 0.001. \end{aligned} \quad (2.1)$$

For each wheatear, we calculated its lean body mass to account for between-individual variation. Variation in wing length of robins was not correlated with their lean body mass (mean = 14.2 g; standard deviation = ± 0.06 g; *adj. R*² = 0.04, *n* = 35, *p* = 0.11). Thus, we assigned 14.2 g as the general lean body mass for all robins.

Table 1. Results of the linear mixed effect models on the changes of evening energy stores between day 3 and day 4 during spring migration. Means and 95% credible intervals (CrI) are given for fixed factors included in the model. 95% CrI not including zero are given in bold. Reference category for night is 'day 3'. ' n_{birds} ' represents the number of birds so that the total sample size for each model was twice as large (experimental day 3 and 4).

	European robin				northern wheatear			
	$n_{\text{birds}} = 28$		$n_{\text{birds}} = 26$		$n_{\text{birds}} = 26$		$n_{\text{birds}} = 25$	
	5°C to 25°C		25°C to 5°C		5°C to 25°C		25°C to 5°C	
	mean	95% CrI	mean	95% CrI	mean	95% CrI	mean	95% CrI
intercept	0.20	−0.16/0.56	0.09	−0.25/0.45	−0.11	−0.39/0.15	0.43	0.12/0.74
experimental day 4	0.34	0.12/0.56	0.57	0.38/0.77	0.15	−0.06/0.38	0.34	0.17/0.51

(e) Statistical analyses

The statistical analyses were implemented using R, v. 3.6.1 [36]. The continuous variables 'evening energy stores' and 'day of the year' (1 January = 1) were scaled and centred (z-transformed) before modelling for each species and each season. We used linear mixed-effects models (LMMs) and generalized mixed-effects models (GLMMs) run with functions in the R-package 'lme4' [37]. Models of main results are described below, for others see supplementary electronic material. Visual inspection of residual diagnostic plots did not show deviation from the model assumptions in all models [38,39].

The change in evening energy stores (response variable, continuous, z-transformed) was analysed using LMMs assuming normally distributed errors for each treatment, species and season. The initial models included the following fixed factor: experimental night (categorical; two levels: night 3, night 4; depending on the treatment, this represents the temperature rise or drop). Bird ID was implemented as a random factor (intercept) to account for the non-independence of repeated measures from the same individual.

The change in the amount of nocturnal migratory restlessness (response variable) was analysed using GLMMs assuming binomially distributed errors separately for each treatment, species and season. The response variable consisted of a two-column matrix with the number of active 1-min intervals per night (success) versus the number of inactive 1-min intervals per night (failure). The model included the following fixed factors: experimental night (categorical, two levels: night 3, night 4; depending on the treatment, this represents the temperature rise or drop), evening energy store (continuous, z-transformed) and day of the year (continuous, z-transformed). The two-way interaction between the experimental night and evening energy store was considered additionally. If it was not significant, it was removed from the final model. Bird ID was included as a random factor (intercept). We added an observation-level random term as a random factor to all GLMMs to correct for overdispersion [40].

We used Bayesian methods in order to assess the uncertainty of the model estimates and model predictions and to obtain uncertainty estimates of the model parameters [38]. Improper prior distributions, namely $p(\beta) \sim 1$ for the coefficients and $p(\sigma) \sim 1/\sigma$ for the variance parameters in all models were used. To obtain posterior distributions of the respective models, we directly simulated 2000 values from the joint posterior distributions of each models' parameters applying the function 'sim()' of the R-package 'arm' [41]. The means of the simulated values from the joint posterior distributions of each model's parameters were used as the respective model estimates and the 2.5%- and 97.5%-quantiles as limits for the 95% credible intervals (CrI). We declared an effect to be significant if the corresponding 95% CrI does not

include zero or if the 95% CrIs of the compared groups do not overlap.

3. Results

(a) Daily food intake, evening energy stores and change in temperature

Daily food intake had a positive significant effect on daily change in energy stores in all groups (electronic supplementary material, table S1 and figure S5). In spring, robins experiencing the temperature drop significantly increased their food intake from day 3 to day 4, while wheatears significantly decreased their food intake when experiencing the temperature rise (electronic supplementary material, table S2 and figures S6 and S7). Otherwise, we found no effect in spring (electronic supplementary material, table S2). In autumn, we found no effect of a change in temperature on daily food intake, except for robins, which experienced the temperature rise that significantly reduced their food intake from day 3 to day 4 (electronic supplementary material, table S2 and figures S6 and S7).

In spring, there was a significant increase in energy stores from evening three to four in all groups except for the wheatears experiencing the temperature rise (table 1 and figure 2; electronic supplementary material, table S3 and figures S8 and S9). In autumn, we found no change in the evening energy stores of robins (table 2; figure 3) but a significant increase in energy stores from evening three to four in both wheatear groups (table 2 and figure 3; electronic supplementary material, table S3 and figures S8 and S9).

(b) Nocturnal migratory restlessness, evening energy stores and change in temperature

In spring, the amount of migratory restlessness significantly increased with the temperature rise in both species (table 3 and figure 4; electronic supplementary material, table S4 and figures S10 and S11). In wheatears, this effect was associated with a significant two-way interaction between the temperature rise and the evening energy stores, suggesting that wheatears with higher evening energy stores were more likely to depart than leaner birds (table 3; electronic supplementary material, figure S12). The evening energy stores only had a significant positive effect on the amount

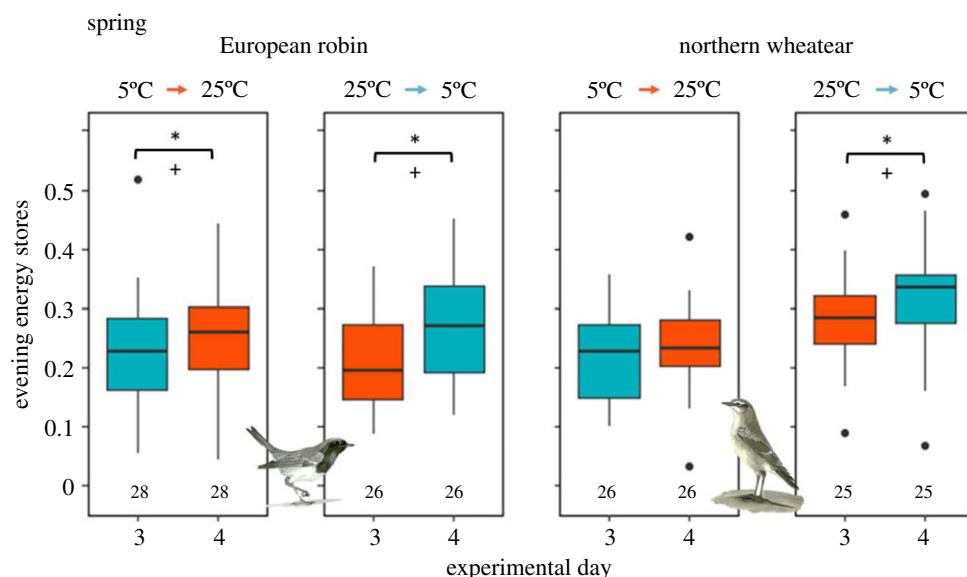


Figure 2. Evening energy stores in European robins and northern wheatears were calculated using evening body mass for day 3 and 4 during spring migration. Blue boxes represent the temperature of 5°C, orange boxes the temperature of 25°C. Significant effects (table 1) are marked with an asterisk. The plus indicates the direction of the effect. The number of birds are given above the x-axis. Box plots show the 5th, 25th, 50th, 75th and 95th percentile as well as outliers (dots). For data of all nights see the electronic supplementary material. (Online version in colour.)

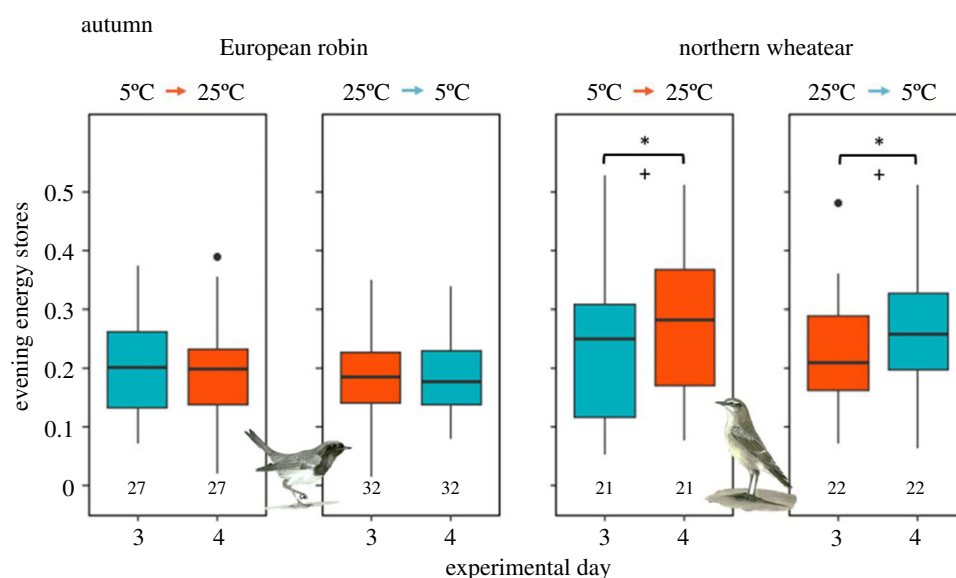


Figure 3. Evening energy stores in European robins and northern wheatears were calculated using evening body mass for day 3 and 4 during autumn migration. Blue boxes represent the temperature of 5°C, orange boxes the temperature of 25°C. Significant effects (table 2) are marked with an asterisk. The plus indicates the direction of the effect. The number of birds are given above the x-axis. Box plots show the 5th, 25th, 50th, 75th and 95th percentile as well as outliers (dots). For data of all nights see the electronic supplementary material, table S3 and figure S7. (Online version in colour.)

Table 2. Results of the linear mixed effect models on the changes of evening energy stores between day 3 and day 4 during autumn migration. Means and 95% credible intervals (CrI) are given for fixed factors included in the model. 95% CrI not including zero are given in bold. Reference category for night is 'day 3'. ' n_{birds} ' represents the number of birds so that the total sample size for each model was twice as large (experimental day 3 and 4).

	European robin				northern wheatear			
	$n_{\text{birds}} = 27$		$n_{\text{birds}} = 32$		$n_{\text{birds}} = 21$		$n_{\text{birds}} = 22$	
	5°C to 25°C		25°C to 5°C		5°C to 25°C		25°C to 5°C	
	mean	95% CrI	mean	95% CrI	mean	95% CrI	mean	95% CrI
intercept	−0.03	−0.39/0.31	−0.32	−0.58/−0.08	0.11	−0.42/0.62	−0.01	−0.44/0.40
experimental day 4	−0.15	−0.36/0.06	0.12	−0.08/0.32	0.32	0.09/0.58	0.31	0.16/0.46

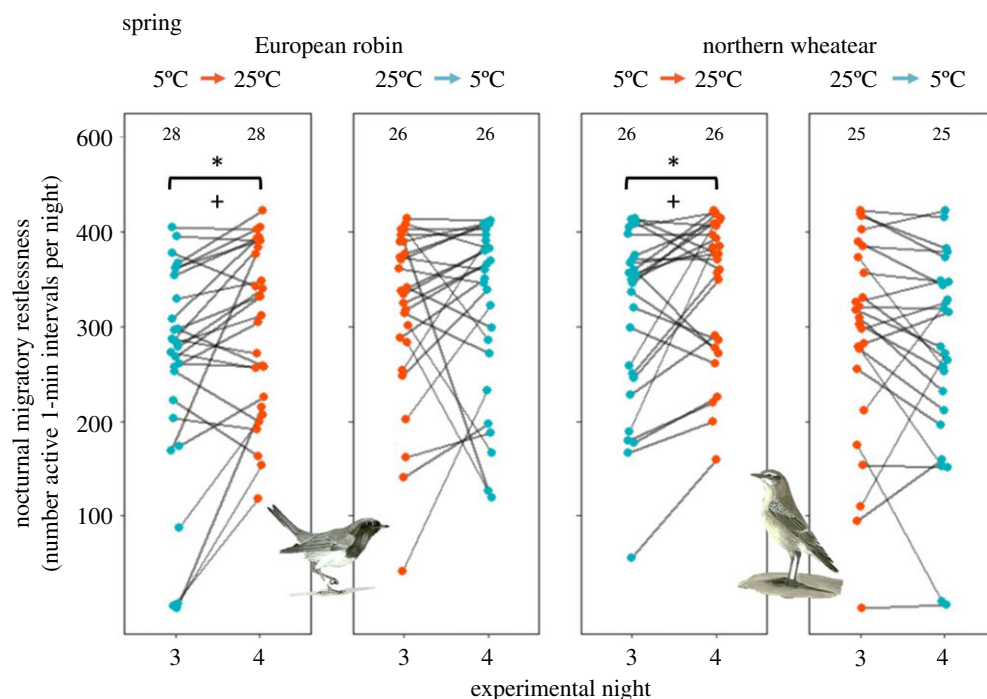


Figure 4. Effects of a change in temperature on migratory restlessness in European robins and northern wheatears for experimental night three and four during spring migration. Migratory restlessness is expressed as the number of active 1 min intervals per night, which was restricted to the first 10 h (see Methods). Blue dots represent the temperature of 5°C, orange dots the temperature of 25°C and are connected for each individual by a line. The number of individuals are given at the top. Significant effects (table 3) are marked with an asterisk. The plus indicates the direction of the effect. For data of all nights see the electronic supplementary material, table S4 and figure S8. (Online version in colour.)

Table 3. Results of four generalized linear mixed effects models explaining the variation in amount of nocturnal migratory restlessness of European robins and northern wheatears between day 3 and day 4 during spring migration. Means and 95% credible intervals (CrI) are given for fixed factors included in the model. 95% CrI not including zero are given in bold. Reference category for night is 'night 3'. ' n_{birds} ' represents the number of birds so that the total sample size for each model was twice as large (experimental day 3 and 4).

	European robin				Northern wheatear			
	$n_{\text{birds}} = 28$		$n_{\text{birds}} = 26$		$n_{\text{birds}} = 26$		$n_{\text{birds}} = 25$	
	5°C to 25°C		25°C to 5°C		5°C to 25°C		25°C to 5°C	
	mean	95% CrI	mean	95% CrI	mean	95% CrI	mean	95% CrI
intercept	0.18	−0.35/0.75	1.38	0.89/1.85	1.29	0.20/3.47	1.01	0.15/1.85
night 4	0.67	0.14/1.20	−0.05	−0.62/0.50	0.75	0.22/1.26	−0.51	−1.08/0.04
energy stores	0.70	0.26/1.14	0.34	−0.13/0.83	−0.26	−1.00/0.56	0.33	−0.46/1.33
day of year	−0.44	−89/0.05	−0.23	−0.67/0.21	0.28	−0.18/0.74	0.03	−0.75/0.84
night 4 × energy stores	NA	NA	NA	NA	1.06	0.18/1.94	NA	NA

of migratory restlessness in robins experiencing the temperature rise (table 3). The temperature drop did not have a significant effect on the amount of migratory restlessness in either species (table 3).

In autumn, we found no effect of the temperature changes on the amount of migratory restlessness in robins (table 4 and figure 5). In wheatears, the temperature rise had a significant negative effect on the amount of migratory restlessness and the temperature drop a significant positive effect (table 4 and figure 5). The evening energy stores only had a significant positive effect on the amount of migratory restlessness in robins experiencing the temperature drop (table 4).

4. Discussion

Our experimental study shows how a change in air temperature affects the departure probability of a medium- and long-distance migrant songbird during spring and autumn migration. In spring, a 20°C temperature rise, which would indicate an acceleration of the advancement of spring, significantly increased the estimated departure probability in both species. Individuals which adjust their decision-making process immediately to temperature changes will be able to shorten their stopover durations and reach higher speeds of migration [42,43]. Higher migration speeds will, in turn, advance breeding

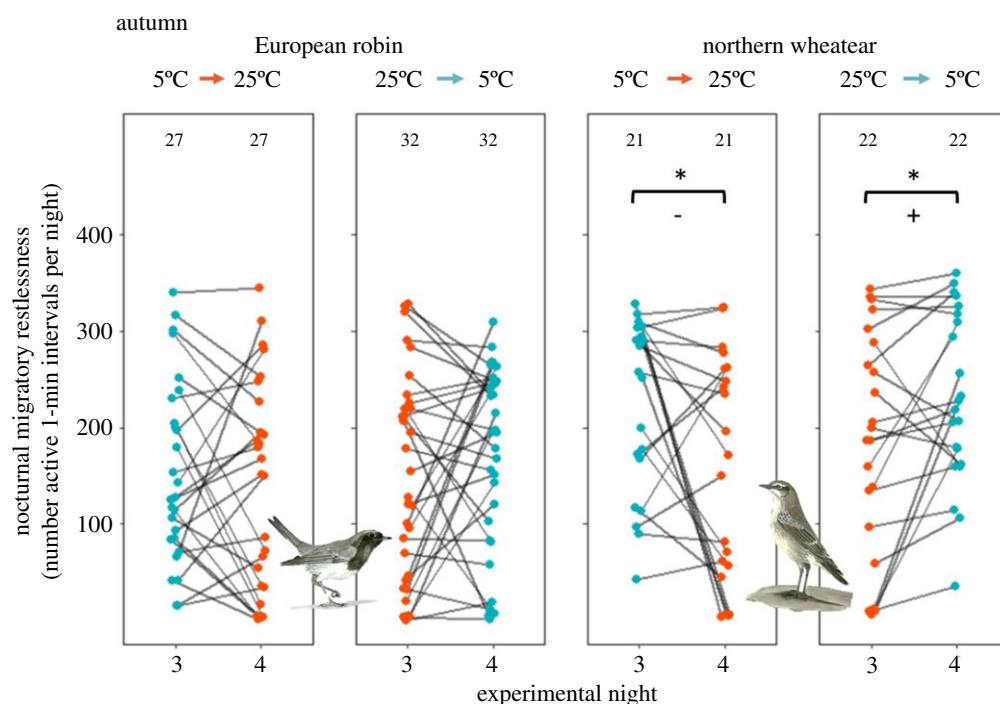


Figure 5. Effects of a change in temperature on migratory restlessness in European robins and northern wheatears for experimental night three and four during autumn migration. Migratory restlessness is expressed as the number of active 1 min intervals per night, which was restricted to the first 7 h (see Methods). Blue dots represent the temperature of 5°C, orange dots the temperature of 25°C and are connected for each individual by a line. The number of individuals are given at the top. Significant effects (table 4) are marked with an asterisk. The plus or minus indicates the direction of the effect. For data of all nights see the electronic supplementary material, table S4 and figure S8. (Online version in colour.)

Table 4. Results of four generalized linear mixed effects models explaining the variation in amount of nocturnal migratory restlessness of European robins and northern wheatears between day 3 and day 4 during autumn migration. Means and 95% credible intervals (CrI) are given for fixed factors included in the model. 95% CrI not including zero are given in bold. Reference category for night is 'night 3'. ' n_{birds} ' represents the number of birds so that the total sample size for each model was twice as large (experimental day 3 and 4).

	European robin				Northern wheatear			
	$n_{\text{birds}} = 27$		$n_{\text{birds}} = 32$		$n_{\text{birds}} = 21$		$n_{\text{birds}} = 22$	
	5°C to 25°C		25°C to 5°C		5°C to 25°C		25°C to 5°C	
	mean	95% CrI	mean	95% CrI	mean	95% CrI	mean	95% CrI
intercept	−0.36	−1.07/0.29	−0.51	−1.15/0.12	0.59	−0.12/1.27	−0.08	−0.87/0.63
night 4	−0.51	−1.22/0.19	0.24	−0.45/0.94	−1.29	−2.29/−0.25	0.96	0.24/1.66
energy stores	0.51	−0.13/1.17	0.83	0.15/1.47	0.11	−0.34/0.57	0.22	−0.50/0.90
day of year	−0.05	−0.61/0.53	0.1	−0.08/0.93	−0.09	−0.64/0.41	−0.32	−1.05/0.41

area arrival timing in comparison to others [44], which often increases fitness [26]. We found no effect of temperature changes on the departure probability of a medium-distance migrant during autumn migration. By contrast, a temperature rise reduced the departure probability of a long-distance migrant while a temperature drop increased it. As such, our results clearly demonstrate that a change in temperature is an important extrinsic departure cue for the decision-making process in migrant songbirds at a stopover site.

One limitation of our study design was that we did not consider time-matched control groups. We therefore cannot completely exclude that the 3 days at a temperature of 5°C or 25°C would induce a spontaneous change in migratory restlessness on day 4. However, previous

restlessness studies did not reveal significant changes in the magnitude of migratory restlessness on day 4 after the initial increase under controlled conditions [22,23]. Consequently, future studies should include temperature controls throughout the experiment, which would probably support the conclusions of this study.

(a) Spring migration

Temperature rise at stopover in spring may signal to a migrant (among others): (i) an improvement of the local feeding conditions through better access to the food sources and/or higher densities/activities of these food sources (e.g. arthropods [16,45]); (ii) improved meteorological conditions

(e.g. wind and cloud cover [25]); and (iii) an accelerated advancement of spring, which increases the probability that environmental conditions en route and at the breeding area have improved as well (e.g. due to earlier snowmelt [24]). The departure probabilities of both robins and wheatears increased after a temperature rise in spring (table 3 and figure 4). Their responses were not affected by variation in food availability because of the *ad libitum* feeding conditions. The fact that their behavioural response occurred demonstrates a causal effect of temperature on the decision-making process during a stopover. Hence, a rise in temperature is an important departure cue increasing the urge to resume migration in spring. Further experiments are, however, required to assess whether more natural temperature changes cause corresponding responses as in this proof-of-concept study. Migrant birds responding to a rise in temperature adjust their progress of migration to the spatially and temporally advancing spring [25] and ultimately optimize the arrival timing at their breeding area depending on the temperature-related environmental conditions [46]. At the same time, the decision-making process is influenced by, among others, the feeding conditions [22]. As variation in ambient temperature is 'inextricably' linked to the feeding conditions under natural conditions, the interplay of both has a significant effect on the departure decision of free-flying migrants. As such, tracking studies provide correlative evidence that free-flying geese reacted sensitively to the progress of the vegetation growth rate ('green wave hypothesis' [47,48]) and/or to the temperature development ('growing degree days' [7,8]) for fine-tuning both the progress of migration and the breeding area arrival timing to the expected future environmental conditions [46]. Because the songbirds studied here are mainly insectivorous, rather than herbivorous (such as the geese), they probably do not follow the 'green wave' or the 'growing degree days', but probably track the seasonal increase in arthropod abundance which is also related to temperature [49].

We found no effect of a temperature drop on the migratory restlessness (table 3 and figure 4), although this drop must have increased the resting metabolic rate [13] and consequently, food intake. The higher energy demands should influence the decision-making process to leave the current location in case of food shortage to find a more favourable site [4]. In our study, the birds counterbalanced the increased energy demands by feeding more mealworms (only significant in robins; electronic supplementary material, table S2 and figures S6 and S7) so that they successfully increased their energy stores despite the lower temperature (table 1 and figure 2). The favourable feeding conditions in our experiment strongly counteracted against departure (figure 4). Thus, the outcome of this interplay of the temperature drop as an extrinsic condition and food intake and energy store as intrinsic conditions did not increase the departure probabilities (figures 3 and 4). This seems advantageous because the spatio-temporal autocorrelation between the environmental conditions at the current location and the breeding area is deteriorated by retreating further away from the migratory destination and thus, making the optimal timing for resuming migration and arriving at the breeding area less well predictable. This supports the notion that landscape movements, *sensu lato* 'reverse migration', are driven by unfavourable feeding conditions probably caused by adverse weather [4,50], as opposed to by a drop in temperature *per se*, though more research is

required to understand the motivation for and function of such movements.

(b) Autumn migration

During autumn migration, birds leave their breeding areas and stopover sites, which become increasingly unfavourable over time in terms of food abundance and weather conditions, and travel towards more benign regions [51]. Migratory birds experiencing favourable feeding conditions and a sudden rise in the temperature, that makes the energy accumulation more efficiently through decreased costs of thermoregulation [13], should therefore stay at the stopover to exploit such suitable sites, which might be rarely encountered during autumn migration, through intensively fuelling [28].

While robins did not show a response to the experimentally changed temperature (table 4 and figure 5), wheatears responded in terms of their nocturnal migratory restlessness (table 4 and figure 4), as predicted and as observed in free-flying wheatears [12]. When experiencing a rise in temperature, the wheatears indeed dropped their amount of migratory restlessness (table 4 and figure 5), and increased their energy stores accordingly (table 2 and figure 3) despite the not significantly affected food intake (electronic supplementary material, table S2 and figures S6 and S7). While carrying surplus energy is costly in terms of energy expenditure [52] and a reduced escape performance [53], it is also beneficial because it prolongs the total flight duration without accumulating further energy, minimizes the risk of starvation [54] and increases, for instance, the time available for anti-predator behaviour [27], and is therefore often observed in wild migratory birds [4]. As such, our experimental results provide causal evidence for a large body of correlative studies and the prediction that a temperature rise in combination with favourable feeding conditions prolongs stopover duration and is a beneficial strategy in autumn.

In response to a temperature drop, wheatears increased their migratory restlessness, i.e. departure probability (table 4 and figure 5), as predicted. A sudden temperature drop is likely to be more persistent and more adverse in autumn than in spring. Instead of waiting for local weather conditions to improve, it seems advantageous to immediately resume migration towards the wintering grounds where more favourable environmental conditions generally persist. At the same time, the birds avoid the locally increased thermoregulation costs due to the lower temperatures [14], shorten the remaining migration distance and approach more suitable stopover sites.

The reasons why the robins did not adjust their migratory restlessness as predicted for autumn (table 4 and figure 5) are currently unknown. As robins pass Helgoland later in autumn (September–November) and winter further north (middle to southern Europe) than wheatears (August–October; sub-Saharan Africa; electronic supplementary material, figures S1 and S3), one could speculate that robins are better adapted to low temperatures than wheatears or to 'waiting out' low-temperature fronts in autumn and winter. If this were the case, the low temperature of 5°C could potentially not have been low enough to stimulate a similar response to that of the wheatears.

The temperature drop and hence the increased energy demands [13,14] did not lead to a significant increase in food intake in both species (electronic supplementary material, table S2 and figures S6 and S7). Despite that, evening energy stores were not affected or even increased

in the wheatears (table 2 and figure 3). There are at least three non-mutually exclusive potential reasons for this discrepancy. First, the amount of fed mealworms was overestimated on day 3 due to many out-thrown mealworms. Second, birds significantly decreased their diurnal activity, despite the increased energy demand, possible through the *ad libitum* feeding conditions. Third, songbirds fed increasingly less, the longer they stopped-over [1,55]. The reduced food intake (electronic supplementary material, figures S6 and S7) could therefore be related to such feeding behaviour and less to the temperature drop.

5. Conclusion

In our proof-of-concept study, we demonstrate for the first time a causal effect of temperature changes on the departure probabilities of medium- and long-distance migrant birds in spring and autumn. This means that temperature change is an important extrinsic departure cue for the decision-making process at a stopover site. Future experiments should measure food intake more precisely and record the bird's diurnal activity as well as its metabolic rate to better disentangle how these three factors interact and jointly affect together with a temperature change the bird's departure probability. Additionally, time-matched control groups with birds kept constantly at the appropriate temperatures are required to demonstrate that the change in temperature and not the duration of captivity affects the bird's departure probability. Although applying smaller, gradual and thus more realistic temperature changes would require a far higher sample size to find an effect, the benefit would be to better predict what kind of temperature changes would affect the departure probability under natural conditions if feeding conditions were constant. Adjusting our proof-

of-concept study to such more natural conditions could substantially improve models of migratory stopover behaviour and predicted impacts of future temperature changes on migration. Warmer temperatures advance migration phenology and arrival timing during spring migration [56]. In addition to a general temperature rise, climate change is associated with increased temperature variation and more frequent extreme temperatures [57]. Migrant birds are therefore in jeopardy when they adjust for instance their breeding area arrival timing to unseasonably warm temperatures en route but experience a significant drop in ambient temperature shortly after arrival, which may lead to delayed or immediate fitness consequences.

Ethics. Experimental procedures were approved by the Ministry of Energy Transition, Agriculture, Environment, Nature and Digitalization (MELUND) Schleswig-Holstein, licence number V244-4829/2017 (33-3/17). All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Data accessibility. All restlessness data used for this study are made available in the additional file: electronic supplementary material complete dataset.xlsx and electronic supplementary material.docx.

Authors' contributions. H.S. conceived the study. T.K. performed the experiment and collected the data. T.K. and H.S. analysed the data. T.K. and H.S. wrote the manuscript.

Competing interests. We declare we have no competing interests.

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References

- Delingat J, Dierschke V, Schmaljohann H, Mendel B, Bairlein F. 2006 Daily stopovers as optimal migration strategy in a long-distance migrating passerine: the Northern Wheatear. *Ardea* **94**, 593–605.
- Ferretti A, Rattenborg NC, Ruf T, McWilliams SR, Cardinale M, Fusani L. 2019 Sleeping unsafely tucked in to conserve energy in a nocturnal migratory songbird. *Curr. Biol.* **29**, 2766–2772. (doi:10.1016/j.cub.2019.07.028)
- Eikehaar C, Winslott E, Hessler S, Isaksson C. 2020 Oxidative damage to lipids is rapidly reduced during migratory stopovers. *Funct. Ecol.* **34**, 1215–1222. (doi:10.1111/1365-2435.13540)
- Schmaljohann H, Eikehaar C. 2017 How do energy stores and changes in these affect departure decisions by migratory birds? – A critical view on stopover ecology studies and some future perspective. *J. Comp. Physiol. A* **203**, 411–429. (doi:10.1007/s00359-017-1166-8)
- Müller F, Taylor PD, Sjöberg S, Muheim R, Tsvey A, Schmaljohann H. 2016 Towards a conceptual framework for explaining variation in the nocturnal departure time of songbird migrants. *Mov. Ecol.* **4**, 24. (doi:10.1186/s40462-016-0089-2)
- Shamoun-Baranes J, Liechi F, Vansteelant WMG. 2017 Atmospheric conditions create freeways, detours and tailbacks for migrating birds. *J. Comp. Physiol. A* **203**, 509–529. (doi:10.1007/s00359-017-1181-9)
- Bauer S, Gienapp P, Madsen J. 2008 The relevance of environmental conditions for departure decision changes en route in migrating geese. *Ecology* **89**, 1953–1960. (doi:10.1890/07-1101.1)
- van Wijk RE, Kölzsch A, Kruckenberg H, Ebbinge BS, Müskens GJDM, Nolet BA. 2012 Individually tracked geese follow peaks of temperature acceleration during spring migration. *Oikos* **121**, 655–664. (doi:10.1111/j.1600-0706.2011.20083.x)
- Xu F, Si Y. 2019 The frost wave hypothesis: how the environment drives autumn departure of migratory waterfowl. *Ecol. Indic.* **101**, 1018–1025. (doi:10.1016/j.ecolind.2019.02.024)
- Schmaljohann H, Korner-Nievergelt F, Naef-Daenzer B, Nagel R, Maggini I, Bulte M, Bairlein F. 2013 Stopover optimization in a long-distance migrant: the role of fuel load and nocturnal take-off time in Alaskan northern wheatears (*Oenanthe oenanthe*). *Front. Zool.* **10**, 26. (doi:10.1186/1742-9994-10-26)
- Schmaljohann H, Fox JW, Bairlein F. 2012 Phenotypic response to environmental cues, orientation and migration costs in songbirds flying halfway around the world. *Anim. Behav.* **84**, 623–640. (doi:10.1016/j.anbehav.2012.06.018)
- Schmaljohann H, Lisovski S, Bairlein F. 2017 Flexible reaction norms to environmental variables along the migration route and the significance of stopover duration for total speed of migration in a songbird migrant. *Front. Zool.* **14**, 17. (doi:10.1186/s12983-017-0203-3)
- Maggini I, Bairlein F. 2013 Metabolic response to changes in temperature in northern wheatears from an arctic and a temperate populations. *J. Avian Biol.* **44**, 1–7. (doi:10.1111/j.1600-048X.2012.05785.x)
- Wikelski M, Tarlow EM, Raim A, Diehl RH, Larkin RP, Visser GH. 2003 Costs of migration in free-flying songbirds. *Nature* **423**, 704. (doi:10.1038/423704a)
- Nuijten RJM, Kölzsch A, van Gils JA, Hoyer BJ, Oosterbeek K, de Vries PP, Klaassen M, Nolet BA. 2014 The exception to the rule: retreating ice front makes Bewick's swans *Cygnus columbianus bewickii*

- migrate slower in spring than in autumn. *J. Avian Biol.* **45**, 113–122. (doi:10.1111/j.1600-048X.2013.00287.x)
16. Avery MI, Krebs JR. 1984 Temperature and foraging success of Great Tits *Parus major* hunting for spiders. *Ibis* **126**, 33–38. (doi:10.1111/j.1474-919X.1984.tb03661.x)
 17. Berchtold A, Nightingale I, Vandermeer C, MacDougall-Shackleton SA. 2017 Experimental temperature manipulations alter songbird autumnal nocturnal migratory restlessness. *Anim. Migr.* **4**, 1. (doi:10.1515/ami-2017-0001)
 18. Gwinner E. 1996 Circadian and circannual programmes in avian migration. *J. Exp. Biol.* **199**, 39–48.
 19. Eikenaar C, Klinner T, Szostek KL, Bairlein F. 2014 Migratory restlessness in captive individuals predicts actual departure in the wild. *Biol. Lett.* **10**, 20140154. (doi:10.1098/rsbl.2014.0154)
 20. Haest B, Hüppop O, van de Pol M, Bairlein F. 2019 Autumn bird migration phenology: a potpourri of wind, precipitation and temperature effects. *Glob. Change Biol.* **25**, 4064–4080. (doi:10.1111/gcb.14746).
 21. Packmor F, Klinner T, Woodworth BK, Eikenaar C, Schmaljohann H. 2020 Departure decisions in songbirds following different migration strategies: long-distance migrants depart earlier and more independently of weather conditions than medium-distance migrants. *Mov. Ecol.* **8**, 6. (doi:10.1186/s40462-020-0193-1)
 22. Klinner T, Buddemeier J, Bairlein F, Schmaljohann H. 2020 Decision-making in migratory birds at stopover: an interplay of energy stores and feeding conditions. *Behav. Ecol. Sociobiol.* **74**, 10. (doi:10.1007/s00265-019-2784-7).
 23. Eikenaar C, Bairlein F. 2014 Food availability and fuel loss predict Zugunruhe. *J. Ornithol.* **155**, 65–70. (doi:10.1007/s10336-013-0987-7)
 24. Jensen RA, Madsen J, O'Connell M, Wisz MS, Tømmervik H, Mehlum F. 2008 Prediction of the distribution of Arctic-nesting pink-footed geese under a warmer climate scenario. *Glob. Change Biol.* **14**, 1–10. (doi:10.1111/j.1365-2486.2007.01461.x)
 25. Haest B, Hüppop O, Bairlein F. 2018 The influence of weather on avian spring migration phenology: what, where and when? *Glob. Change Biol.* **24**, 5769–5788. (doi:10.1111/gcb.14450)
 26. Kokko H. 1999 Competition for early arrival in birds. *J. Anim. Ecol.* **68**, 940–950. (doi:10.1046/j.1365-2656.1999.00343.x)
 27. Dierschke V. 2003 Predation hazard during migratory stopover: are light or heavy birds under risk? *J. Avian Biol.* **34**, 24–29. (doi:10.1034/j.1600-048X.2003.03049.x)
 28. Alerstam T, Lindström Å. 1990 Optimal bird migration: the relative importance of time, energy, and safety. In *Bird migration: physiology and ecophysiology* (ed. E. Gwinner), pp. 331–351. Berlin, Germany: Springer.
 29. Svensson L. 2001 Identification of western and eastern olivaceous, booted and syke's warblers. *Bird. World* **14**, 192–219.
 30. Eikenaar C, Hessler S, Fischer S, Bairlein F. 2019 An exception to the rule: captivity does not stress wild migrating northern wheatears. *Gen. Comp. Endocr.* **275**, 25–29. (doi:10.1016/j.ygcen.2019.02.010)
 31. Maggini I, Bairlein F. 2010 Endogenous rhythms of seasonal migratory body mass changes and nocturnal restlessness in different populations of Northern Wheatear *Oenanthe oenanthe*. *J. Biol. Rhythm* **25**, 268–276. (doi:10.1177/07487304.10373442)
 32. Berthold P, Fiedler W, Querner U. 2000 Die Zugunruhe bei Vögeln: eine Darstellung nach Videoaufnahmen bei Infrarotlichtbeleuchtung. *J. Ornithol.* **141**, 285–299. (doi:10.1007/BF02462238)
 33. Jenni L, Jenni-Eiermann S. 1998 Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521–528. (doi:10.2307/3677171)
 34. Guglielmo CG, McGuire LP, Gerson AR, Seewagen CL. 2011 Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *J. Ornithol.* **152**, 75. (doi:10.1007/s10336-011-0724-z).
 35. Kelsey N, Bairlein F. 2019 Migratory body mass increase in Northern Wheatears (*Oenanthe oenanthe*) is the accumulation of fat as proven by quantitative magnetic resonance. *J. Ornithol.* **160**, 389–397. (doi:10.1007/s10336-018-1621-5)
 36. Team RC. 2019 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
 37. Bates D, Mächler M, Bolker B, Walker S. 2014 lme4: Linear mixed-effects models using Eigen and S4. R package version 11-7. See <http://CRAN.R-project.org/packages=lme4>.
 38. Korner-Nievergelt F, Roth T, von Felten S, Guélat J, Almasi B, Korner-Nievergelt P. 2015 *Bayesian data analysis in ecology using linear models with R, BUGS, and stan*. London, UK: Elsevier.
 39. Hartig F. 2017 Package 'DHARMA' residual diagnostics for hierarchical (multi-level/mixed) regression models. (Version 0.1.3. ed). See <https://cranr-project.org/web/packages/DHARMA/DHARMA.pdf>.
 40. Harrison XA. 2014 Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**, e616. (doi:10.7717/peerj.616)
 41. Gelman A, Hill J. 2007 *Data analysis using regression and multilevel/hierarchical models*. Cambridge, UK: Cambridge University Press.
 42. Schmaljohann H. 2018 Proximate mechanisms affecting seasonal differences in migration speed of avian species. *Sci. Rep.* **8**, 4106. (doi:10.1038/s41598-018-22421-7)
 43. Schmaljohann H, Both C. 2017 The limits of modifying migration speed to adjust to climate change. *Nat. Clim. Change* **7**, 573. (doi:10.1038/nclimate3336)
 44. Tøttrup AP, Klaassen R.H.G., Kristensen MW, Strandberg R, Vardanis Y, Lindström Å, Rahbek C, Alerstam T, Thorup K. 2012 Drought in Africa caused delayed arrival of European songbirds. *Science* **338**, 1307. (doi:10.1126/science.1227548)
 45. Meyer GA, Senulis JA, Reinartz JA. 2016 Effects of temperature and availability of insect prey on bat emergence from hibernation in spring. *J. Mammal.* **97**, 1623–1633. (doi:10.1093/jmammal/gyw126)
 46. Lameris TK, van der Jeugd HP, Eichhorn G, Dokter AM, Bouten W, Boom MP, Litvin KE, Ens BJ, Nolet BA. 2018 Arctic geese tune migration to a warming climate but still suffer from a phenological mismatch. *Cur. Biol.* **28**, 2467–2473. (doi:10.1016/j.cub.2018.05.077)
 47. van der Graaf S.A.J., Stahl J, Klimkowska A, Bakker JP, Drent RH. 2006 Surfing on a green wave – how plant growth drives spring migration in the Barnacle Goose *Branta leucopsis*. *Ardea* **94**, 567–577.
 48. Shariati NM *et al.* 2015 Satellite- versus temperature-derived green wave indices for predicting the timing of spring migration of avian herbivores. *Ecol. Indic.* **58**, 322–331. (doi:10.1016/j.ecolind.2015.06.005)
 49. Danks HV. 1999 Life cycles in polar arthropods: flexible or programmed? *Eur. J. Entomol.* **96**, 83–102.
 50. Nilsson C, Sjöberg S. 2016 Causes and characteristics of reverse bird migration: an analysis based on radar, radio tracking and ringing at Falsterbo, Sweden. *J. Avian Biol.* **47**, 354–362. (doi:10.1111/jav.00707)
 51. Newton I. 2008 *The migration ecology of birds*. London, UK: Academic Press.
 52. Klaassen M, Lindström Å. 1996 Departure fuel loads in time-minimising migrating birds can be explained by the energy costs of being heavy. *J. Theor. Biol.* **183**, 29–34. (doi:10.1006/jtbi.1996.0198)
 53. Kullberg C, Fransson T, Jakobsson S. 1996 Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proc. R. Soc. Lond B* **263**, 1671–1675. (doi:10.1098/rspb.1996.0244)
 54. Pravosudov VV, Lucas JR. 2001 Daily patterns of energy storage in food-caching birds under variable daily predation risk: a dynamic state variable model. *Behav. Ecol. Sociobiol.* **50**, 239–250. (doi:10.1007/s002650100361)
 55. Fransson T. 1998 Patterns of migratory fuelling in Whitethroats *Sylvia communis* in relation to departure. *J. Avian Biol.* **29**, 569–573. (doi:10.2307/3677177)
 56. Haest B, Hüppop O, Bairlein F. 2020 Weather at the winter and stopover areas determines spring migration onset, progress, and advancements in Afro-Palearctic migrant birds. *Proc. Natl Acad. Sci. USA* **117**, 17 056–17 062. (doi:10.1073/pnas.1920448117)
 57. Rahmstorf S, Coumou D. 2011. Increase of extreme events in a warming world. *Proc. Natl Acad. Sci. USA* **108**, 17 905–17 909. (doi:10.1073/pnas.1101766108)