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RESEARCH ARTICLE

Energetics and fuel use vary with migration strategy across populations of Common Blackbirds

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Abstract

1. Seasonal migration is a physiologically demanding endeavour that animals prepare for by finding and storing energy. Species and populations vary in their tendencies to migrate and the energetic demands of migration likely determine the degree and type of preparation that different animals undergo.
2. Birds fuel their migratory flight using stored fat. Previous work has suggested that in addition to being energetically dense, certain lipids like polyunsaturated fatty acids (PUFAs) may be particularly efficient fuels that are preferentially mobilized for flight or serve may additional functions, such as modulating membrane fluidity or stimulating gene expression as ligands, during long-distance migratory flight.
3. Using a series of behavioural and metabolic experiments in a partial transplant aviary setting of Common Blackbirds (Turdus merula) populations that range from being fully migratory to partially migratory to sedentary, we asked how migratory restlessness (*Zugunruhe*), weight gain and oxidation of stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6) and alpha linolenic acid (18:3n-3; ALA) varied with migratory mode and season.
4. We found no population-level differences in the timing of *Zugunruhe*. However, before migration, a greater proportion of individuals from fully migratory populations exhibited weight gain and migratory restlessness, as well as increased oxidation of PUFAs. After *Zugunruhe*, only individuals from migratory populations showed reduced PUFA oxidation. All populations showed increased oxidation of saturated fat after *Zugunruhe*.

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5. Our results demonstrate that certain migratory traits, like the timing of *Zugunruhe* are expressed similarly across populations in response to local environmental conditions, while other traits, like lipid oxidation, can vary among populations.

KEYWORDS
Common Blackbird, fatty acids, migratory restlessness, stable isotopes

1 | INTRODUCTION

Migration is often an endurance event that requires sufficient fuel. While human athletes that fail to eat optimally before endurance competitions may cost themselves a chance at medaling or qualifying for future events, wild animals that fail to fuel properly before undertaking migrations face reduced fitness from missed breeding opportunities or starvation (e.g. Biebach, 1985; Brodersen et al., 2008). Just as humans vary in their exercise regimes, wild species vary enormously in their propensity and capacity to migrate as well as in their migratory modes which range from diel vertical migration in pelagic zooplankton (Lampert, 1989) to annual circumpolar migration in terns (Egevang et al., 2010). Because the costs and benefits of migration vary spatiotemporally across habitats and species' ranges (e.g. Alerstam & Lindström, 1990; Brodersen et al., 2014), individuals and populations within species also vary in their propensity to migrate and the distances that they migrate (Adriaensen & Dhont, 1990; Kaitala et al., 1993; Linek et al., 2021; Lundberg, 1988; Zúñiga et al., 2017). These diverse types of migration across and within species require different amounts and forms of energy (e.g. Bairlein, 1990; Berthold, 1984; Biebach et al., 1986).

Regardless of their total migration distance, birds primarily use fat to fuel migration and previous studies suggest that fat provides approximately 90% of the energy used in long-distance flights (Bairlein, 2002; Bairlein & Gwinner, 1994; Guglielmo, 2018; Jenni & Jenni-Eiermann, 1998; McWilliams et al., 2004; Ramenofsky, 1990). Compared with proteins or carbohydrates, lipids contain eight times the energy per unit of wet mass, making stored lipids the most efficient fuel source for migrating birds (Jenni & Jenni-Eiermann, 1998). Birds appear to take advantage of the energy density of lipids prior to migration and during migratory stopovers by accumulating large amounts of fat, often nearly doubling their body mass due to fat gain (Bairlein, 2003; Guglielmo, 2018). Previous studies suggest that migrating birds alter their energy intake and dietary composition, providing evidence that they have both metabolic as well as behavioural control over the amount and composition of lipids that they accumulate (Bairlein, 1990; Bairlein & Gwinner, 1994; Lindström, 1991). Numerous studies have documented hyperphagia during the migratory period (reviewed in Bairlein, 1990; Bairlein & Gwinner, 1994) as well as increased food utilization efficiency, both of which result in increased fat assimilation (e.g. Bairlein, 1987). Studies also suggest that the degree of fat gain is related to migration distance and feeding opportunities *en route*, such that species that migrate further need to accumulate more fuel to complete their migration (Bairlein, 2003; Hedenstrom & Alerstam, 1997).

During migration and long-distance flight, omega-3 and omega-6 polyunsaturated fatty acids (n-3 and n-6 PUFAs), in particular linoleic acid (18:2n-6; LIN) and alpha linolenic acid (18:3n-3; ALA), may be particularly important as readily oxidizable fuel sources (Guglielmo, 2010, 2018; Price, 2010). For example, studies have found that birds, including those measured during migration, are able to mobilize a greater proportion of PUFA, which have multiple double bonds, compared with monounsaturated fatty acids (MUFAs) or saturated fatty acids (SFAs) and that birds mobilize fatty acids with shorter carbon chain at higher rates compared with those of longer chain lengths (Price & Guglielmo, 2009; Price et al., 2011). Early studies specifically on PUFA use by birds suggested that migratory shorebirds may seek out high n-3 PUFA foods during stopover (Maillet & Weber, 2006), potentially improving their flight performance (i.e. natural doping) through increased muscle oxidative capacity (Maillet & Weber, 2007). Findings from subsequent laboratory studies in which birds were fed specific diets provide more mixed findings about the relative roles of n-3 versus n-6 fatty acids as well as the exact mechanisms through which such fats might enhance flight performance (e.g. Dick & Guglielmo, 2019a, 2019b; McWilliams et al., 2020; Nagahuedi et al., 2009; Price, 2010; Price & Guglielmo, 2009). For instance, a recent study by McWilliams et al. (2020) found that during endurance flight, European starlings *Sturnus vulgaris* expend 11% less energy when fed diets containing more of the n-6 and n-3 PUFA LIN and ALA compared with diets containing more of the MUFA oleic acid (18:1n-9), but birds consuming more n-6 PUFA also experienced greater oxidative damage. In contrast, Dick and Guglielmo recently found that dietary n-3 altered muscle phospholipid fatty acid composition and decreased muscle oxidative capacity in yellow-rumped Warblers *Setophaga coronata* but that dietary PUFA content and composition (i.e. n-3 vs. n-6) did not lead to differences in flight performance (2019a) or oxidative damage (2019b). Consequently, additional integrative work is needed to understand how migratory birds metabolize different fatty acids.

Species that vary in migratory propensity across their range may provide a unique opportunity for understanding how seasonal shifts in lipid metabolism are related to migratory preparations. Here, we examined how songbirds select and use different fatty acids before and after migratory restlessness (i.e. *Zugunruhe*) and how lipid metabolism varies with migration strategy across populations within a single species. We studied different populations of Common Blackbirds *Turdus merula* because blackbirds are a model system that vary widely in their migration strategies (Biebach, 1977; Lundberg, 1985; Schwabl, 1983), thereby permitting a partial transplant experimental design. Populations in eastern Poland and northern Russia are fully migratory; populations in Germany are partially migratory; and populations in Spain and southern France are fully resident (Linek et al., 2021). This unique system in which birds from northern Russia, southwest Germany and southern France were fed identical diets and experienced the same environmental conditions, such as day length and temperature, in southwest Germany allowed us to identify population-level differences metabolism independently of geographic variation in resources (i.e. food availability and nutritional composition) and environmental cues. Our aviary populations of blackbirds permitted us to ask how migratory versus sedentary lifestyles may increase or relax selection for fat storage and oxidation of specific fats.

Before and after the autumn migratory season, we orally administered ^{13}C-labelled stearic acid (18:0), oleic acid (18:1n-9), LIN (18:2n-6) or ALA (18:3n-3) tracers to birds and then measured their rates of oxidation via ^{13}C enrichment of CO_2 from breath samples (McCue, 2011; McCue & Welch, 2016). This method allowed us to determine the extent to which the birds were oxidizing the different ^{13}C-labelled compounds as fuel, resulting in more of the ^{13}C-labelled compounds being exhaled in breath. We selected these fatty acids because several of them make up substantial proportions of pre-migratory deposited fat in birds and are thought to be important dietary fuel sources for birds during migration (e.g. Bairlein & Gwinner, 1994; Pierce & McWilliams 2005, 2014) and because this suite of 18-carbon compounds, which included an SFA, MUFA, n-6 PUFA and n-3 PUFA, allowed us to explore effects of unsaturation and double bond position (Price et al., 2008, 2011). To understand how fat accumulation and use varied over autumn migration, we fed birds ^{13}C-labelled compounds and collected samples for breath $^{13}CO_2$ analyses twice: (1) after the breeding season, but prior to the onset of *Zugunruhe* (September) and (2) at the end of migratory period (late October), which we determined based on radio telemetry for freely moving individuals within large outdoor aviaries. We also measured individual body mass and resting metabolic rates before and after migratory restless.

We predicted that the relative rates of oxidization of fatty acids differing in desaturation and number of double bonds would vary based on a combination of season and migration strategy. Based on previous work (e.g. Price et al., 2008), we expected that fatty acid oxidation rates across all populations would generally increase with the degree of desaturation and the number of double bonds. PUFAs are thought to be particularly efficient fuels for migratory flight in songbirds (e.g. McWilliams et al., 2020) and because migratory blackbirds have been shown to have higher levels of PUFA compared with residents (Jensen et al., 2020). Therefore, we expected that birds from migratory populations would vary more in PUFA (i.e. LIN and ALA) oxidation compared with either SFA or MUFA oxidation between sampling periods. We also predicted that birds from fully migratory populations would oxidize all fats more readily during and after migration compared with prior to *Zugunruhe*, whereas birds from fully resident populations would differ less in oxidation based on season.

2 | MATERIALS AND METHODS

2.1 | Capture and aviary conditions

Wild Common Blackbirds were caught with mist nets in Malinki, Russia (55.45821°N, 37.17912°E) between 2013 and 2015, in Radolfzell, Germany (47.77355°N, 9.04902°E) in 2015 and 2018, and Bollène, France (44.27589°N, 4.72177°E) in 2014, 2015 and 2018 (Table S1). Capture and transfer of French birds was approved under permit DDTM34-2019-06-10454 by the Direction départmentale des territoires et de la mer (DDTM). Capture of German birds was approved under permits 35-9185.81/G-17/109 and 35-9185.81/G-20/95 by the Regierungpräsidium Freiburg. Capture of Russian birds was approved following §44 and §6 of the Federal Law of the Russian Federation No. 52 from 24 April 1995, and we obtained permits for the utilization (withdrawal) of wildlife objects which are not listed as game species No. BC-02-04/32-5935 (26 April 2013) from the Federal Service for Supervision of Natural Resources (Rosprirodnadzor), No. 2 (4 November 2014) and No. 4 (18 March 2015) from the Ministry of Ecology and Nature Management of Moscow District. Wild Russian birds were quarantined for 40 days before being transferred from Russia to Germany from the Animal Disease Control Station of the Southern Administrative District of Moscow (Sovetsky District Veterinary Hospital). Export of birds from Russia was approved under permits No. FC/UVN-01/57843 (26 June 2013), FC/UVN-01/93276 (06.05.2014), FC/AP01/127093 (20 April 2015) by the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor). During quarantine, the analysis of faecal samples for infectious agents (Ornithosis [DNA Chlamydophila psttaci], Influenza virus type A RNA, Salmonellosis, Helminths) was carried out at the City Veterinary Laboratory of the Moscow Veterinary Association (veterinary and sanitary inspection reports No. 193482, 193510, 16428/5, 16428/1, 16428/4). Captured birds were transported to our aviary facility in Radolfzell, Germany (47.7646°N, 8.9961°E). Additional Russian and French blackbirds were bred in our aviary facility between 2014 and 2017 (Table S1) and used in our experiment in 2020. After blackbirds were transferred to our aviary facilities, they were paired and housed in semi-natural breeding aviaries (L × B × H in m: 3.5 × 2.5 × 2.5). They received a mixture of food: live mealworms *Tenebrio molitor*, earthworms *Dendrobena* spp.; frozen fly larvae *Alphitobius diaperinus*; pellets including Orlux Uni Pate Premium, Orlux Insect Pate Premium, Country's Best Floating Micro, Nutribird T16 (all previous from Versele-Laga) and Granivit pellets (Chemivit); vitamins and minerals (Korvimin, Vitakalk) and water *ad libidum*.

2.2 | Measurements of activity

The migratory restlessness (*Zugunruhe*) of each individual in the study was determined using continuous radio telemetry with an automated receiving unit (ARU, Sparrow Systems Fisher) attached to mounted H-antennas (ATS) within the outdoor aviary facility. We fitted 60 blackbirds (*n* = 20 Russian; *n* = 20 German; *n* = 20 French individuals; 10 male/female pairs; Table S1) with a radio transmitter (≤2.6 g; frequencies within the 165 mHz band, pulse width: 20 ms, pulse interval: 3600 ms; Sparrow Systems Fisher) by means of a leg loop harness. Two ARUs searched for frequencies of deployed radio transmitters every 240 s, recording values for signal and noise. ARU A searched for 28 and ARU B search for 32 radio transmitters. The ARUs were placed so that no signals of pre-programmed frequencies were missed. This enabled the activity of individuals to be monitored on a near continuous basis. We quantified activity by comparing the change in signal strength between subsequent received recordings using a 4 dB threshold (see Zúñiga et al., 2016 for details of calibration and general procedures of the ARU System). Raw data were visually inspected for artefacts and for evidence of malfunctioning transmitters. The processed data provided a binary estimate of whether a bird was active approximately every 4 min throughout the study. Individual radio transmitters were deployed approximately a month before expected *Zugunruhe* in early September (Partecke & Gwinner, 2007; Zúñiga et al., 2016) and remained on individuals until early-December after the autumn migration period.

We characterized the timing of *Zugunruhe* using complementary methods. First, we calculated the proportion of time the individual spent active during a 2-h period, between the hour after sunset and hour before sunrise as used in previous studies (Partecke & Gwinner, 2007; Zúñiga et al., 2017) using the binary method described above. We then looked for changes in average nocturnal activity by implementing changepoint analysis (Killick & Eckley, 2014; Van Doren et al., 2017), a penalized method that identifies changes in the mean and variance of time series data. Changes in nocturnal activity were then identified where there was more than one changepoint in the time series for each individual, which previous studies have used as evidence for periods of elevated nocturnal activity (Helm et al., 2019; Van Doren et al., 2017). Using the changepoints, we were able to then determine the period of peak restlessness in a consistent manner across populations. Individuals without evidence of *Zugunruhe* were noted and were used to calculate the percentage of individuals within a population that expressed *Zugunruhe*. To estimate confidence intervals for the proportion of individuals in a population exhibiting *Zugunruhe*, we used a non-parametric bootstrap.

2.3 | Dosing experiment and respirometry

We captured adult aviary blackbirds (*n* = 24 Russian; *n* = 24 German; *n* = 24 French individuals; Table S1) with hand nets for the combined metabolic /^{13}C-labelled fatty acid experiment at or before sunrise during the pre−/early migratory period ('before *Zugunruhe*') and the late/post-migratory period ('after *Zugunruhe*') (Figure 1a,b: Date early: 9 September 2020 to 20 September 2020; late: 22 October 2020 to 2 November 2020). The same 36 individuals were used in both experimental periods but were provided a different fatty acid tracer during each period. Individuals were captured before they had been fed their daily meal by animal caretakers and before they had a chance to forage in outdoor aviaries. Because blackbirds in our aviaries are typically housed as pairs, we captured and used both birds within a pair on the same day to avoid repeatedly disturbing the same pair and to minimize the social stress of a partner being removed. After capture, birds were immediately placed in cloth bags and then transported to an adjacent indoor laboratory. In the laboratory, each individual was weighed to the nearest 0.1 g using an Ohaus Scout Pro STX balance.

2.4 | Measurement of metabolic rates

Our experiment involved simultaneously measuring metabolic while sampling exhaled breath to calculate the oxidative rates of different fatty acids. During each tracer/ respirometry trial, 6 (3 males, 3 females) birds were placed individually in 1400-mL chambers through which fresh air was pumped at a rate of 150–200 mL min^{-1}, which allowed $FeCO_2$ within the chamber to reach ~2% without inducing hypercapnia. The chambers were maintained at thermoneutral temperatures (28 ± 1°C) in the dark to minimize additional activity. Rates of oxygen consumption (V_{O2}) and carbon dioxide (V_{CO2}) production were sampled from consecutive chambers for 120 s per cycle over the course of an 180-min trial between 8:00 and 11:00 h using a multiplexed, push mode respirometry system (Sable Systems International). This multiplexing schedule resulted in each individual being sampled for 120 s every 14 min (2 min per individual × 6 individuals + 2 min baseline). The incurrent air was from an outside source and air flow into the metabolic chambers was scrubbed of water vapour using CO_2-equilibrated Drierite (White et al., 2006) and then the excurrent gas samples measured for water vapour (Sable Systems, RH-300). The excurrent sample was then scrubbed of water vapour using CO_2 equilibrated Drierite before CO_2 measurement and then CO_2 was removed using a combination of soda-lime and Ascarite II (Arthur H. Thomas Co., Sigma Aldrich #223913) before O_2 sample measurement. V_{O2} and V_{CO2} were calculated as mL/min^{-1} using the following equations (9.11 and 9.12, Lighton, 2018):

$$V_{O_2} = V_E\left(\frac{Fi_{O_2} - Fe_{O_2}}{1 - Fi_{O_2}}\right),$$

$$V_{CO_2} = V_E\left(Fi_{CO_2} - Fe_{CO_2}\right),$$

where V_E is the temperature-corrected flow rate in mL/min^{-1}, Fi_{O_2} and Fi_{CO_2} are the fractional incurrent concentrations of O_2 and CO_2, and Fe_{O_2} and Fe_{CO_2} are the fractional excurrent concentrations from the respirometry chamber. Both O_2 and CO_2 values were zeroed and spanned against reference lab gases (scrubbed 0% CO_2: 21.00% O_2 Heliox mixture and 1.96% CO_2: 98.4% Nitrogen reference, Linde AG) before both stages of the experiment (Linde AG).

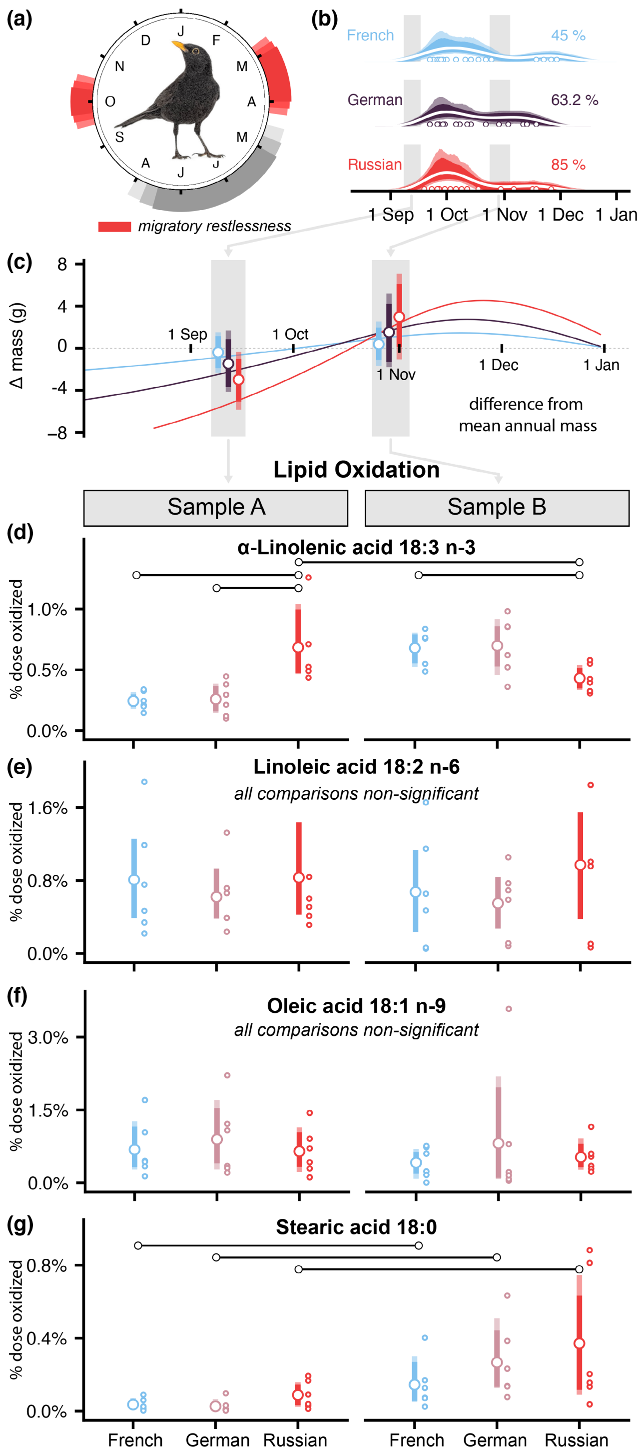

FIGURE 1 Timing of seasonal migratory restlessness and associated traits. (a) Timing of Common Blackbird spring and autumn migratory restlessness (*Zugunruhe*) in relation to other life history stages such as reproduction. (b) Results from automated activity recordings to determine the timing and percentage of individuals in a population expressing *Zugunruhe*. The percentages are the percentage of individuals determined by changepoint analysis (see methods). The timing of the before and after *Zugunruhe* lipid oxidation experiment (Sample A and B, before and after respectively) are depicted in grey. (c) Change in mass during each of the lipid oxidation experiments (shown in grey) relative to standardized average annual mass. The curve is the population level change fitted using a Generalized Additive Model with cyclic cubic spline fit. (d–g) Percent of the total lipid dose oxidized during each trial. The left figures correspond with the before *Zugunruhe* period (Sample A, grey arrows at top of subfigure d) whereas the right subfigures correspond with the post *Zugunruhe* period (Sample B). The small points are raw data (individual samples) and the error bars are 95% and 99% confidence intervals for that population and period. The large point within the error bars is the population level mean. Ties depict $p < 0.05$ in post-hoc pairwise comparisons.

2.5 | Measurement of fatty acid tracer oxidation

After acclimating birds to the respirometry chamber for 15 min, we used a 20-mL syringe (Poulten und Graf GmbH, Fortuna Series, DE) to collect a background breath sample by drawing excurrent gas samples into the syringe at approximately 60 mL min^{-1} and then injecting them into helium-evacuated 12-mL-vacutainer vials (Labco Limited). After taking the background breath sample, we orally gavaged adult individuals from each of the three populations (Table S1) a ^{13}C-labelled bolus of 50 mg (Cambridge Isotope Laboratories, Inc; USA) of either stearic acid (SA, 18:0), oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6) or alpha-linolenic acid (ALA, 18:3n-3) suspended in 0.5 mL of sunflower oil via a syringe with an attached 1.5 × 50 mm gavage needle (McCue et al., 2010). Each individual received only one labelled compound per sampling period ('before *Zugunruhe*' or 'after *Zugunruhe*'). Previous studies have shown that no measurable tracer remains after 30 days (McCue et al., 2010). After labelled lipid administration, individuals were returned to their respirometry chamber and respired gas was collected in Exetainers every 15 min (over a period of 3 h) using the procedure as described above. Thereafter, individuals were then returned to their aviary and provided water and food ad libitum.

The δ^{13}C of the CO$_2$ in each vial was analysed using a HeliFANPlus nondispersive infrared spectrometer (Fischer, Analysen Instrumente GmbH; Germany) interfaced with a FANas autosampler as previously described (McCue, Guzman, et al., 2015; McCue, Passement, et al., 2015; Plasman et al., 2019). Internal calibrations were conducted before each round of ($n = 60$) unknown samples using the built-in standards provided by the manufacturer. Additionally, sample vials containing gases with known ^{13}C values (hereafter: laboratory standards) were run in triplicate every 30 samples to detect and correct for any minor baseline drift. These laboratory standards consisted of two cylinders of custom-blended gases with ^{13}C values representing natural abundance (i.e. −36‰) and artificial enrichment (i.e. 495‰) as previously verified on multiple aliquots by the University of Arkansas Stable Isotope Laboratory. The ^{13}C values were reported in terms of δ^{13}C and expressed in units '*per mil*' (‰ Vienna Pee Dee Belmnite ($_{VPDB}$) where the ratio of ^{13}C to ^{12}C is 0.0112372).

We quantified the net rates of ^{13}C tracer oxidation in units of moles/min^{-1} by using the rate of CO$_2$ production from the flow through respirometry coupled with the tracer-induced increase in $\delta^{13}C_{CO2}$. We then calculated the atom fraction excess (AFE) using the following equation from Welch et al. (2016):

$$\mathrm{AFE}=\left[\frac{\mathrm{VPDB}\times\left(\left(\frac{\delta^{13}\mathrm{C}_{\mathrm{enriched}}}{1000}\right)+1\right)}{1+\left(\mathrm{VPDB}\times\left(\frac{\delta^{13}\mathrm{C}_{\mathrm{enriched}}}{1000}\right)+1\right)}\right]-\left[\frac{\mathrm{VPDB}\times\left(\left(\frac{\delta^{13}\mathrm{C}_{\mathrm{background}}}{1000}\right)+1\right)}{1+\left(\mathrm{VPDB}\times\left(\frac{\delta^{13}\mathrm{C}_{\mathrm{background}}}{1000}\right)+1\right)}\right],$$

where $\delta^{13}C_{\mathrm{enriched}}$ refers to the $\delta^{13}C$ in breath samples after labelled tracer exposure and $\delta^{13}C_{\mathrm{background}}$ is the $\delta^{13}C$ prior to tracer administration. We then calculated the instantaneous molar rate of tracer oxidation, T using the following equation from Welch et al. (2016):

$$T=\left[\frac{V_{\mathrm{CO}_2}\times\mathrm{AFE}}{m\times\theta\times\mathrm{RF}\times K}\right],$$

where m is the tracer molar mass, θ is the quantity of ^{13}C atoms in the tracer, retention factor (RF; assumed to be 0.86) is the term that corrects for the loss of ^{13}C label to other carbon pools, and K is the volume of CO_2 produced per mg of tracer oxidized. Values of K were determined based on the stoichiometry of substrate oxidation and the volume occupied per mole of CO_2, per the following equation:

$$K=\frac{(C\times V)}{M},$$

where C is the number of carbon atoms per tracer, V is the volume of 1 mole of gas at standard temperature and pressure (STP: ~22.4 L) and M is the molar mass of the tracer. Cumulative tracer oxidation over the duration of the experiment was calculated by fitting a one-dimensional numerical integral via spline interpolation across the 180-min measurement period and summing the area under the curve in the R package 'MESS'.

2.6 | Body mass and condition

As part of respirometry measurements and ^{13}C-labelled fatty acid dosing, we measured body mass of all birds in September and October. The body mass was also measured again in December as part of routine husbandry. To investigate changes in mass between our two measurement periods, we normalized our values against the annual average body mass for each individual.

2.7 | Statistical analyses

2.7.1 | Timing of *Zugunruhe*

To determine whether there were differences in the timing of *Zugunruhe* between individuals, we modelled the relationship between activity as a response variable using a Gaussian linear model and the interaction between the three populations, sex, mean body mass and whether the birds were wild-caught or aviary-bred (Table S1). We then performed pairwise post-hoc comparisons by estimating the marginal least-square means for each group (modality of sex population) using R.

2.7.2 | Changes in body mass

To determine changes in body mass between our two sampling periods, we modelled the relationship between mass changes using a Gaussian linear model with an interaction between populations and sex. We then performed pairwise post-hoc comparisons by estimating the marginal least-square means for each group.

2.7.3 | Energetics

To understand if there were individual-, sex- or population-level differences in energetics 'before' and 'after *Zugunruhe*' period, we modelled the resting metabolic rate using a linear mixed-effects model (LMM) with body mass, sex, season and population as fixed predictors and individual identity as a random effect. We then performed pairwise post-hoc comparisons by estimating the marginal least-square means for each group.

2.7.4 | Lipid oxidation

We tested for differences in percent of ALA, LA, OA and SA dose oxidized and peak oxidation rate using generalized LMMs with a Gamma error structure and a log link. The percent dose oxidized and peak oxidation rates (*nmols*) was modelled as the response with body mass, population of origin, and sample date as fixed effects and individual ID as a random effect. We then performed pairwise post-hoc comparisons by estimating the marginal least-square means for each group. We also tested for different in global differences between different tracers in total lipid oxidation and peak oxidation rate using a generalized linear model as described above but with tracer type, body mass and sample date as predictors. We also performed pairwise post-hoc comparisons by estimating the marginal least-square means for each group.

3 | RESULTS

3.1 | Differences in *Zugunruhe* behaviour between populations

The timing of *Zugunruhe* spanned 3 weeks with a trend of the German females being the earliest (28 September, 95% CI 24 September to 22 October) and the German males the latest (19 October 95% CI 9 October to 4 November). The median date of activity for all populations was 9 October (Figure 1a,b). However, there was no evidence that the timing of *Zugunruhe* differed by populations, sex or body mass (LM, $F_{11,45}=1.206$, $p=0.31$, Adj. $R^2=0.04$) and no post-hoc comparisons were significant. We also did not find any evidence that the timing of *Zugunruhe* differed when accounting for population, sex, origin (i.e. wild-caught or aviary-bred) or change in mass (LM, $F_{11,45}=1.309$, $p=0.25$, Adj. $R^2=0.06$), and no post-hoc comparisons were significant. However, the proportion of individuals undergoing *Zugunruhe* varied by population, with fully migratory Russian population having greatest proportion, intermediate in the German, and the least in the French population (*proportion observed Zugunruhe* via *non-parametric bootstrap*: Russian 0.85, 95% CI 0.62–0.95, German 0.63, 95% CI 0.40–0.81, French 0.45, 95% CI 0.25–0.66). Post-hoc pairwise comparisons of estimated marginal means found that only contrasts between Russian and French populations were significant (Russian—French, odds ratio = 0.144, $p=0.03$).

3.2 | Body mass

Between the September period before *Zugunruhe* and the late October period after *Zugunruhe*, both male and female Russian blackbirds gained significantly more weight than Germans of either sex, with Russian females gaining the most weight of any sex by population combinations (Figure 1c; *change in mass [g]*, RU males 5.10, 95% 2.72–7.48, RU females 6.79, 95% CI 4.41–9.18, DE males 0.45, 95% CI $^{-}$1.93–2.83, DE females 1.08, 95% CI = $^{-}$1.30–3.47). Both contrasts were significant (Males RU—DE, $t_{65}=2.75$, $p<0.05$, Females RU—DE, $t_{65}=3.38$, $p<0.05$). In addition, Russian females put on significantly more mass than French females (Males FR—DE, $t_{65}=2.35$, $p<0.05$).

3.3 | Seasonal changes in resting metabolic rate

There were no significant differences in whole organism resting metabolic rates (Table S2) between populations or sexes (LMM, Population: $F_{1,65.56}=0.63$, $p=0.53$, Sex: $F_{1,65.57}=3.57$, $p=0.063$) or in mass-specific metabolic rate either between sexes or populations (LMM, Population: $F_{1,65.56}=1.68$, $p=0.20$, Sex: $F_{1,65.55}=1.67$, $p=0.19$). There were no differences in whole organism or mass-specific metabolic rates before *Zugunruhe* or after *Zugunruhe* between populations nor were any pairwise post-hoc comparisons significant.

3.4 | Global differences in fatty acid oxidation

Across fatty acid types, individuals oxidized significantly less of the stearic acid dose compared with oleic, LIN or ALA prior to *Zugunruhe* (cumulative oxidation %: stearic 0.03% dose, 95% CI 0.02%–0.05%; oleic 0.59% dose, 95% CI 0.38%–0.92%; linoleic 0.53% dose, 95% CI 0.33%–0.83%; alpha-linolenic 0.40% dose, 95% CI 0.26%–0.63%). All three comparisons were significant in post-hoc comparisons using the Kenward–Roger method (cumulative oxidation %: stearic—oleic, $z=9.066$, $p<0.05$; stearic—linoleic, $z=8.98$, $p<0.05$; stearic—alpha-linolenic, $z=7.78$, $p<0.05$). Similarly, in the second round of measurements post *Zugunruhe*, individuals oxidized significantly less of the stearic acid dose when compared LIN and ALA but not oleic (cumulative oxidation %: stearic 0.03% dose, 95% CI 0.02%–0.05%; linoleic 0.61% dose, 95% CI 0.39%–0.95%; alpha-linolenic 0.57% dose, 95% CI 0.37%–0.87%). These two comparisons were significant in post-hoc comparisons using the Kenward–Roger method (cumulative oxidation %: stearic—linoleic, $z=3.678$, $p<0.05$; stearic—alpha-linolenic, $z=3.47$, $p<0.05$). There was no significant between body mass and the total amount oxidized ($t=-1.224$, $p=0.221$). There was a non-significant decreasing trend between the percent of the dose oxidized in 180 min and degree of unsaturation from oleic to LIN to ALA.

There was a similar pattern in the maximal observed oxidation rates between fatty acids, wherein the maximal rate of oxidation for stearic acid was lower compared with oleic, LIN or ALA both prior to *Zugunruhe* (maximal oxidation rate: stearic 0.86 nmol, 95% CI 0.53–1.4 nmol; oleic 11.67 nmol, 95% CI 7.39–18.46 nmol; linoleic 9.84 nmol, 95% CI 6.16–15.73 nmol; alpha-linolenic 5.89 nmol, 95% CI 03.78–9.21 nmol). All three comparisons were significant in post-hoc comparisons using the Kenward–Roger method (cumulative oxidation %: stearic—oleic, $z=7.94$, $p<0.05$; stearic—linoleic, $z=7.67$, $p<0.05$; stearic—alpha-linolenic, $z=5.73$, $p<0.05$). Similarly, in the second round of measurements post *Zugunruhe*, the maximal oxidation rate for the stearic acid dose was lower than LIN and ALA, but not oleic (cumulative oxidation %: stearic 3.9 nmol, 95% CI 2.45–6.24 nmol; linoleic 10.30 nmol, 95% CI 6.60–16.08 nmol; alpha-linolenic 9.01, 95% CI 5.78–14.02 nmol). All three comparisons were significant in post hoc comparisons using the Kenward–Roger method (cumulative oxidation %: stearic—oleic, $z=3.27$, $p<0.05$; stearic—linoleic, $z=3.02$, $p<0.05$; stearic—alpha-linolenic, $z=2.62$, $p<0.05$). There was no significant relationship with body mass and maximal rate of oxidation ($t=-1.379$, $p=0.17$). Similarly, there was a non-significant decreasing trend between the percent of the dose oxidized in 180 min and degree of unsaturation from oleic to LIN to ALA.

3.5 | Percent fatty acid dose oxidized

Russian blackbirds oxidized significantly more of the ALA dose during our first 180-min trial prior to *Zugunruhe* compared with either German or French birds (Figure 1d; *cumulative ALA oxidation [%]*, RU 0.58% dose, 95% CI 0.39–0.88, FR 0.24% dose, 95% CI 0.16–0.36, DE 0.17% dose, 95% CI 0.12–0.26). Both were significant in post-hoc comparisons using Kenward–Roger method (ALA % dose RU—FR, $z=2.859$, $p<0.05$; RU—DE, $z=4.32$, $p<0.05$). However, during the second round of measurements post *Zugunruhe*, we observed the opposite pattern—Russian blackbirds oxidized less of the ALA dose than either the French or German populations (*cumulative ALA oxidation [%]*, RU 0.43% dose, 95% CI 0.29–0.63, FR 0.83% dose, 95%

CI 0.55–1.3, DE 0.65% dose, 95% CI 0.44–0.94). These comparisons were only significant between the Russian and French populations (ALA RU—FR, $z = 2.43$, $p < 0.05$) and not between Russian and German populations (ALA RU—DE, $z = 1.49$, $p = 0.30$). However, Russian blackbirds did not oxidize significantly more ALA before and after *Zugunruhe*, whereas both German and French birds oxidized significantly less of the dose prior to *Zugunruhe* (ALA % dose DE early—late, $z = 10.231$, $p < 0.05$; FR early—late, $z = 8.99$, $p < 0.05$). There was no significant difference before and after *Zugunruhe* between any of the populations in oxidation of the linoleic or oleic acid dose (Figure 1e,f). All three populations of blackbirds oxidized significantly more of the stearic acid dose post *Zugunruhe* than before (Figure 1g; stearic acid % dose: RU early—late, 0.04%–0.24% dose, $z = 4.00$, $p < 0.05$; DE early—late, 0.02%–0.34% dose, $z = 6.34$, $p < 0.05$; FR early—late, 0.02%–0.12% dose, $z = 3.99$, $p < 0.05$). There were no differences between populations within either measurement period and there was not a significant relationship between body mass and percent fatty acid dose oxidized.

3.6 | Peak fatty acid oxidation rate

There was no significant difference in the peak oxidation rate of ALA, LIN or oleic acid when comparing between populations or sampling periods. However, we did find that there was a significant difference in the peak oxidation rate of stearic acid between sampling periods (stearic acid maximal oxidation rate nmol: RU early—late, 1.1–4.2 nmol, $z = 3.76$, $p < 0.05$; DE early—late, 0.5–5.4 nmol, $z = 6.61$, $p < 0.05$; FR early—late, 0.6–2.1 nmol, $z = 3.36$, $p < 0.05$), but not between populations within a sampling period. In none of the models, was there a significant relationship between body mass and peak rate of fatty acid oxidation.

4 | DISCUSSION

In this study, we showed that preparations for migration, including expression of *Zugunruhe*, body weight gain, fatty acid metabolism, vary at the population-level within a single model species for variation in migratory mode under shared environmental conditions. Remarkably, despite having been in captivity for up to 7 years or hatching in captivity (Table S1), a higher proportion of individuals from fully migratory populations went through *Zugunruhe* compared with birds from partially migratory and year-round resident populations. We demonstrated that individuals from fully migratory populations preferentially oxidize less of the n-3 PUFA ALA during the post-migratory period compared with the pre-migratory period even in an aviary in an area where wild populations consist of both migratory and non-migratory individuals. Birds from fully migratory populations also gained the most weight between the pre- and post-migratory periods and the highest proportion of individuals experiencing restlessness (*Zugunruhe*). Together, these findings suggest that compared with birds from non-migratory populations, birds from migratory populations expressed greater weight gain, variation in ALA oxidation and *Zugunruhe* in a seasonal environment.

Our study is the first to demonstrate differences in oxidation rates of a fatty acid among individuals of single species based upon their migration strategy and season. Previous studies in a diversity of migratory and non-migratory birds suggest that n-3 or n-6 PUFA may be especially important for migration (McWilliams et al., 2020; Nagahuedi et al., 2009; Price, 2010; Price & Guglielmo, 2009). For instance, McWilliams et al. (2020) found that Eurasian starlings used less energy during flight when fed diets containing LIN and ALA compared with diets containing more oleic acid. However, they also found that birds consuming diets with more of the n-6 PUFA LIN incurred greater oxidative damage, suggesting that there are costs as well as benefits to PUFA consumption. Other recent studies on migratory songbirds have found little to no benefits from variation in the n-3 and n-6 PUFA content of diet and tissues (Dick & Guglielmo, 2019a, 2019b), suggesting that different species of birds likely vary in their fatty acid metabolism. Here, we found that, even under common aviary conditions, blackbirds from one fully migratory Russian population oxidized similar proportions of the n-6 PUFA LIN across seasons, but more of the n-3 PUFA ALA before the migration season. In contrast, birds of the same species from non-migratory or partially migration populations did not vary in ALA oxidation rates. A recent field-based study also found that migrant blackbird individuals had significantly higher plasma ALA, but not LIN, content compared with residents during the autumn migration season, which the authors suggested was likely the result of either dietary or storage preferences for ALA (Jensen et al., 2020). Interestingly, Jensen et al. (2020) found no differences in the susceptibility of lipids to peroxidation, in terms of fatty acid unsaturation index, between migrants and residents, suggesting that ALA may provide fuel efficiency benefits if birds are able to increase their ALA content without increasing overall susceptibility to lipid peroxidation. Taken together, these recent findings and our own suggest that migratory songbirds can vary in their ALA oxidation rates seasonally, which may allow them to accumulate more ALA during the migration season, thus potentially saving energy during flight without substantially increasing oxidative damage.

Previous studies have found that fatty acid oxidation and mobilization in songbirds increases with carbon chain length and degree of unsaturation, such that PUFA are typically oxidized at higher rates than MUFA, which are themselves oxidized at higher rates than SFA (e.g. Price et al., 2008, 2011). We kept carbon chain length constant at 18, but examined oxidation rates of fatty acids that varied in their degree of unsaturation, finding that birds from all populations oxidized a significantly lower proportion of stearic acid, a SFA, compared with oleic acid, a MUFA or the PUFA LIN and ALA, in line with past work. Unlike previous studies, in which migratory state did not affect relative mobilization (Price et al., 2008, 2011), our finding of seasonal differences in ALA oxidation in Russian blackbirds, suggests that fatty acid mobilization, while small overall (<1% of dose) relative to storage and/or egestion, can shift seasonally. However, across populations we did not find a significant trend in fatty acid oxidation rates based on degree of unsaturation nor were differences between oxidation rates of LIN, an n-6 PUFA, or ALA, an n-3 PUFA, significant. Our findings of similar LIN and ALA oxidation rates when all three populations were analysed together fits with those of Price et al. (2011), who found similar rates of LIN and ALA mobilization at the mitochondrial level across five different species of birds.

Our finding that birds from a fully migratory population went through seasonal shifts in weight gain and fatty acid metabolism in a shared aviary environment is especially intriguing in light of recent evidence that cue thresholds for migration vary across species' ranges (Burnside et al., 2021; Packmor et al., 2020), including in our study system (Linek et al., 2021). Within their native range, birds from fully migratory populations begin their migration at warmer temperatures and longer day lengths compared with individuals from partially migratory populations that migrate (Linek et al., 2021). This geographic variation in cue responses is thought to be a consequence of the typically longer distances that individuals from fully migratory populations travel to reach their wintering grounds (e.g. Russia to Spain or the Baltic Coast) compared with migratory individuals from within partially migratory populations (e.g. southern Germany to southern France or Spain). Our study suggests that even when individuals from fully migratory populations experience conditions and cues during the pre-migratory season that differ from those experienced in their native range, they nevertheless go through similar metabolic preparations for migration. Effect sizes in the wild may be even higher if local conditions cause birds to express traits more strongly (e.g. greater fat accumulation by Russian birds in Russia). Hence, our data confirm that certain behavioural and physiological adjustments relevant for migration seem to be intrinsically hard-wired (Bairlein, 1990; Berthold, 1984; Liedvogel et al., 2011; Merlin & Liedvogel, 2019).

We expected to find higher variance in all measured parameters in individuals from partial migrant populations (i.e. German birds) relative to those from either resident (i.e. French birds) or full migrant (i.e. Russian birds) populations. However, we found that variance was similar across our populations and that individuals from partially migratory populations were more similar to those from resident populations than they were to individuals from fully migratory populations. Individuals that undergo longer, more continuous migratory journeys (i.e. Russian birds) may need to be better prepared than individuals that undergo shorter migration movements, such as those observed in the German population. As migrant individuals from partial migrant blackbird populations have been previously shown to undergo similar metabolic and behavioural shifts as those from migrant populations (Fudickar et al., 2013), it is important to understand the extent and duration of a ‘typical’ migratory movement between populations. By more accurately understanding the demands and benefits of varying migratory strategies of individuals from different populations, we will be able to more firmly place individual differences within the context of fitness.

Certain traits related to migration are likely to be more fixed while others are likely more flexible in response to local cues or are only fixed at the population-level. We found that behavioural traits like the timing of *Zugunruhe* varied little across populations in our aviary while the percentage of individuals exhibiting *Zugunruhe*, weight gain and fatty acid storage varied with season and migratory tendency, suggesting that these traits are less flexible within our study populations. Past researchers have found that even in captivity, migratory species exhibit migratory restlessness or *Zugunruhe* (e.g. Berthold, 1999). Here, we found that autumn *Zugunruhe* was similar in timing in all populations echoing the findings of a previous study of captive birds from a partially migratory population (Partecke & Gwinner, 2007). Although *Zugunruhe* of avian migrants has been related to migratory behaviour in the wild (Berthold, 1984), repeated findings of *Zugunruhe* even in non-migratory bird populations have renewed questions about the interpretation of *Zugunruhe* (Helm & Gwinner, 2006). Our data on *Zugunruhe* appear to confirm these findings: in captivity, some degree of *Zugunruhe* is present within blackbirds across populations regardless of their migratory tendency, but migratory behaviour may be induced more strongly in wild migratory birds that experience additional cues from those experienced in an aviary (e.g. changes in food availability).

The question of whether migration or residency is the ancestral condition for migratory birds remains an active debate (e.g. Berthold, 1999; Rappole et al., 2003; Winger et al., 2019; Zink, 2002). Species that vary in migration tendency across populations provide us with a unique lens thru which to examine the evolution of migration behaviour and the suite of traits associated with this phenotype (Berthold, 1996; Chapman et al., 2011). Our findings that traits such as *Zugunruhe* are present in all contemporary populations despite varying migratory strategies provides supports that migration may be the ancestral condition even in sedentary populations of this species. However, our findings that fatty acid metabolism and weight gain are higher in fully migratory populations offer mixed support: these traits may either have been selected for more recently in these populations or may have been lost in more sedentary populations with more stable food supplies throughout the year. Future studies examining both the metabolic and behavioural migratory preparations (or lack thereof) of additional species that vary in their migratory tendency, such as the aptly named American Robin *Turdus migratorius*, will help us understand of how general our findings are across birds. For example, it remains unclear if the traits that we found to be more consistent across seasonal environments are similarly expressed across species where residency is the dominant strategy and migration is rare, which might either provide support to the argument that migration is the ancestral condition or that overwinter survival in resident areas selects for similar traits. In contrast, if species that are mostly composed of migrants do not express migratory preparations across multiple seasonal environments, this would suggest that migratory preparations may be threshold dependent and labile. With the rapid advancement of technologies that allow research to follow individuals across multiple years, future studies should focus on linking variation in migratory behaviours and associated phenotypic traits with their consequences on fitness.

As migratory animals experience rapid human-induced environmental change, understanding how these traits originate, evolve and are maintained is critical for our understanding of migratory ecology in the future under global change.

AUTHOR CONTRIBUTIONS

Cornelia W. Twining, Jeremy Ryan Shipley, Marshall D. McCue and Jesko Partecke conceived ideas and designed methodology. Ivan Pokrovsky, Cornelia W. Twining, Jeremy Ryan Shipley, Jesko Partecke, Arnaud Gregoire and Bruno Faivre wrote ethics permits and captured birds. Cornelia W. Twining, Jeremy Ryan Shipley, Martin Wikelski and Jesko Partecke contributed funding. Jeremy Ryan Shipley and Marshall D. McCue collected data and Jeremy Ryan Shipley performed analyses. Cornelia W. Twining led writing of the manuscript and all authors contributed to drafts and gave approval for publication.

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CONFLICT OF INTEREST STATEMENT

MDM works for Sable Systems, which makes the respirometry system used in this study.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.ksn02v792 (Twining & Shipley, 2023).

ETHICS STATEMENT

Capture of German birds and research in Germany was approved by the Regierungpräsidium Freiburg under permits 35-9185.81/G-17/109 and 35-9185.81/G-20/95. Capture and transfer of French birds was approved under permit DDTM34-2019-06-10454 approved by the Direction départmentale des territoires et de la mer (DDTM). Capture of Russian birds was approved following §44 and §6 of the Federal Law of the Russian Federation No. 52 from 24 April 1995, and we obtained permits for the utilization (withdrawal) of wildlife objects which are not listed as game species No. BC-02-04/32–5935 (26 April 2013) from the Federal Service for Supervision of Natural Resources (Rosprirodnadzor), No. 2 (4 November 2014) and No. 4 (18 March 2015) from the Ministry of Ecology and Nature Management of Moscow District. Export of birds from Russia was approved under permits No. FC/UVN-01/57843 (26 June 2013), FC/UVN-01/93276 (5 June 2014), FC/AP01/127093 (20 April 2015) by the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Blackbird origins, ages at capture, sexes and experimental treatment

Table S2: Observed metabolic rates during oxidation experiment—measured minimum metabolic rates observed during a continuous 60 s period over the duration of the lipid oxidation experiment. Values are presented as mean ± 1 SD.

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