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Supplementary material

Programmed and flexible: long-term *Zugunruhe* data highlight the many axes of variation in avian migratory behaviour

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SUPPLEMENTARY MATERIAL

APPENDIX 1

SUPPLEMENTARY METHODS

Origin and Maintenance of Birds: Birds originated from the following locations: Austrian stonechats: Lower Austria (48°14'N, 16°22'E; n = 157); Irish stonechats: Iveragh Peninsula near Killarney, in the County of Kerry, Ireland (c. 52°N, 10°W; n = 83); African stonechats (data partly published in Helm and Gwinner 2006): Lake Nakuru region, Kenya (0°14'S, 36°0'E; n = 20), and Mount Meru region, Tanzania (3°50'S, 36°5'E; n = 4), and 1 F1, Kenya x Tanzania; Siberian stonechats: vicinity of Naursum National Park (c. 51.5°N, 63°E; n = 53), Kazakhstan.

Detailed descriptions of breeding and raising conditions have been published elsewhere (Gwinner et al. 1987, Helm 2003, 2009). Briefly, juveniles were taken from their nests while still depending on their parents, either from breeding aviaries or from the wild, and were then hand-raised in Andechs, Germany (48° N, 11° E). These birds had hatched at various times of the breeding period in this multiple-brooded species (Helm 2009). All birds received daily fresh water and a custom-made food mixture including mealworms (Gwinner et al. 1995). They were generally weighed and checked for moult once per week. To obtain detailed information on postjuvenile moult (in US terminology, the first prebasic moult,

Humphrey and Parkes 1959), birds were temporarily checked more frequently (Helm and Gwinner 1999).

Photoperiodic Conditions: The vast majority was kept under conditions that simulated day length experienced by Austrian stonechats around the annual cycle, switching on and off daylight at the beginning and end of civil twilight, respectively. Day length was changed at weekly intervals, and between late September and mid March it was adjusted to simulate migration from a summer latitude of 47.5°N to a winter latitude of 40°N. For the subset of birds exposed to simulated day length experienced by Siberian stonechats, we accommodated for an alternative migration route by simulating wintering areas at 25°N and adjusting day length from early September until early May (for details on these conditions, see Helm et al. 2009).

Activity Recording and Initial Processing: Birds were kept in registration cages that recorded their locomotor activity around the clock via passive infrared detectors (Intellisense XJ-413T; CK Systems; 12m/40°range). In contrast to microswitches, infrared detectors record not only perch hopping, but all spatio-temporal changes of a bird's moving body. Custom-made recorders stored the number of movements per two-minute interval, and we then pooled the data of five consecutive two-minute intervals. Because infrared detectors are highly sensitive, we introduced a threshold to filter out noise. Screening of the data showed that noise patterns were suppressed if values below 20 activity counts per ten minutes were omitted (I. Schwabl-Benzinger, pers. comm.). Therefore, we scored each ten-minute interval as "active" if a total of 20 or more activity counts were registered in the five two-minute intervals. We then extracted the total number of ten-minute intervals with activity during the dark and light fractions of the day, discounting one transitional ten-minute interval in the morning and in the

evening, respectively.

Treatment of Moulting and Missing Data: Earlier observations (Helm 2003) indicated that juvenile stonechats show nocturnal restlessness at early ages, even before postjuvenile moulting, when they are unlikely to commence actual migration. However, young birds can initiate migration during late stages of body moulting (Jenni and Winkler 1994). Accordingly, we attributed early post-fledging nocturnal activity to behavioural development and excluded all data prior to the start of postjuvenile moulting from our analysis (affecting 176 birds). We did allow for overlap of *Zugunruhe* with advanced moulting stages, shown by approximately half of measured birds (Figure 2). In 22 cases, no information on moulting was available, and we left these data unmodified. Our dataset contained a small amount of missing activity data for some birds, generally consisting of periods of a few days, but occasionally several weeks. Because our changepoint-based time series analysis could not handle missing data, we addressed this either by splitting up time series at large gaps (> 7 days missing) or by filling in small gaps with the average value of the two weeks flanking the gap (≤ 7 days missing).

Defining Spring and Autumn Migration Periods: To be maximally inclusive, we used data from all birds throughout the full annual cycle to identify elevated activity periods (i.e. those representing *Zugunruhe*). As migration occurs twice per year, we subdivided the annual cycle into two halves to quantify spring and autumn migration periods separately for each year. We defined spring/autumn cut-off dates separately for each population because of inherent population-level differences in migration timing. For each group, we first calculated the daily mean nocturnal activity level throughout the year. Then, we identified the two 60-day periods between the vernal and autumnal equinoxes with the lowest mean nocturnal activity, corresponding to summer and winter, respectively. The midpoints of these 60-day periods—

when nocturnal activity was at its minimum—delimited the start and end of spring and autumn seasons (see supplementary R script). We calculated the mean activity level in the 30 days surrounding these winter and summer boundary dates as a measure of baseline (i.e. non-migratory) activity. For a given bird, we only analysed migration periods with at least 90 days of activity data.

Changepoint Analysis for Identifying and Characterising Periods of Zugunruhe: For changepoint analysis, we applied the pruned exact linear time (PELT, Killick et al. 2012) algorithm implemented in the “cpt.meanvar” function, with a Poisson test statistic. A changepoint is identified if its addition to the model sufficiently improves the log-likelihood enough to overcome a penalty value used to prevent overfitting (i.e. to prevent too many changepoints from being identified). The Bayesian Information Criterion (BIC) is often used as such a penalty value, and it is proportional to the natural logarithm of the number of data points ($= 2 \cdot \ln(n)$, where n is the number of data points). Because bird activity usually varied considerably, this default penalty value (BIC) often resulted in over 10 changepoints identified per half-year period. Although these corresponded to clear changes in bird activity, we adjusted the default settings and used a more conservative penalty value to select the ~2-3 greatest points of change in activity level during a period, corresponding to the onset and end of *Zugunruhe* and any substantive within-period changes. By trial and error, we determined that multiplying BIC by 18 was appropriate and generally identified the expected number of changes (supplementary Figure 1). Finally, because we expected bouts of *Zugunruhe* to be weeks in duration, we used the “minseglen” argument to prevent successive changepoints from being identified within 15 days of one another. Such settings are flexible and can be adjusted in our script to fit patterns of any focal study species (for details, see supplementary script). For further details, see supplementary R script. In about 1% of migration periods, the

analysis identified non-contiguous elevated segments; these cases were not consistent with our assumption of a single *Zugunruhe* period in a given season. We excluded five of these bimodal cases and retained a subset of data derived from the others when they matched expected patterns. Sometimes, the analysis detected no change in nocturnal activity during a given period; in these cases activity was typically quite low overall, in line with birds that did not engage in *Zugunruhe* at all (supplementary Figure 1D). Rarely, no changepoints were identified but the level of activity was consistently high. These cases generally corresponded to periods for which our data only covered a subset of a migration season, or potentially to aberrant individuals. Because we could not accurately determine these birds' *Zugunruhe* status, we excluded them from the analysis if mean activity was above a threshold, defined as the 95th percentile of mean nocturnal activity from summer and winter neutral periods. In this way, we assigned non-*Zugunruhe* status only to periods for which nocturnal activity levels were consistent with those during summer and winter non-migration periods; the < 2% of analysed migration periods that did not pass this criterion were excluded.

Factors Affecting Occurrence of Zugunruhe: We combined all years after the first because sample size declined greatly after the first year, and because we expected any transition to be most pronounced from the first, naïve year to subsequent migratory seasons with at least one completed migratory journey. We then combined automated stepwise removal of non-important terms by AIC ("stepAIC" function in the MASS package, Venables and Ripley 2002) and manual elimination of non-significant terms not removed in the preceding step. We tested pairwise inter-population differences in the proportion of periods with *Zugunruhe* during spring and autumn, and we corrected for multiple comparisons using the joint distribution of the *z* statistics ("single-step" option in "adjusted" function, package *multcomp*, Hothorn et al. 2008).

Variation in Zugunruhe Timing and Intensity: To achieve a larger and more representative dataset, we included data from all three photoperiods. We separately analysed data from spring and autumn periods. For each timing or intensity metric (e.g., onset, mean, and end dates, duration, mean and peak intensity), we constructed an initial model with population, sex, age, photoperiod, and age \times population interaction as categorical fixed effects and individual bird as a random intercept effect. From this initial model, we manually removed terms that were non-significant. Once a model was solely composed of significant terms, we applied the Tukey method to conduct pairwise tests of significant difference among populations (package multcomp; Hothorn, 2008). When the age \times population interaction was significant (rarely), the multiple comparisons presented in figures use a version of the model without the interaction for clarity and in order to show the overall effect of population. We do describe the interactions in the text when affecting one of the non-hybrid groups (only one case). Similarly, for comparing intensity and duration of *Zugunruhe* between spring and autumn migration periods, we used linear mixed models, beginning with the same fixed and random effects just described and dropping non-significant fixed terms. We manually defined contrasts to test for significance of the effect of season for each population and adjusted for multiple comparisons using the “single-step” option, as above.

Population-wide Nocturnal and Diurnal Activity During the Migration Periods: For each population, we identified the two 60-day periods in the first and second halves of the year with the highest mean nocturnal activity (similar to our procedure for identifying neutral periods) and calculated mean nocturnal and diurnal activity levels for each bird during this period. For each bird, we also used the mean nocturnal activity level for its most active 15-day period in the 60-day window as a measure of peak activity.

We first visually inspected activity profiles during the annual cycle at a population level, comparing the activity profiles between (i) hybrids and their parental populations, (ii) the three migratory populations, and (iii) age groups. We then used linear mixed models to analyse these data in a manner similar to that described for changepoint-derived *Zugunruhe* periods. We compared mean and peak activity levels across all individuals of the stonechat populations regardless of their assigned *Zugunruhe* status.

Covariation of Diurnal and Nocturnal Activity Levels: We analysed the association between nocturnal and diurnal activity levels within individuals, including data from all photoperiods to achieve a larger and more representative dataset. For each migration period, we conducted two tests: we compared mean diurnal activity levels in the 15 days immediately preceding *Zugunruhe* onset to mean diurnal activity in the first 15 days after onset. We then did the same for the end of *Zugunruhe*. In this way, we minimised the possibility that activity differences could be explained by differences in day length at the time when birds showed *Zugunruhe*. We used two linear mixed models, beginning with the fixed effects of time (during *Zugunruhe* or not), sex, population, age, and all interactions; and a random intercept term of individual bird. We then eliminated non-significant terms with the “step” function in the lmerTest package (Kuznetsova et al. 2015) and by manual elimination.

We also studied the association between nocturnal and diurnal activity levels within individuals during both migration seasons and the 30-day neutral summer and winter periods. We used linear mixed models, with activity during each day as the response variable and corresponding nocturnal activity as a fixed continuous predictor. Sex, population, and photoperiod were added as additional fixed effects and iteratively removed if not significant. We included a random intercept term of individual bird (to account for average variation in nocturnal activity level among individuals) and a random slope term (to prevent

pseudoreplication when testing the significance of the fixed predictor). We constructed separate models for each season (autumn and spring *Zugunruhe* periods, and summer and winter neutral periods). Data were centred before modelling to aid convergence; this does not affect fixed effect estimates.

Covariation of Activity Between Migratory and Non-migratory Contexts: Finally, we studied whether variation in mean *Zugunruhe* intensity could explain variation in activity during other times of the year, among individuals. We constructed linear mixed models with a migration period's mean *Zugunruhe* intensity as a fixed predictor and mean diurnal activity level during the adjacent summer or winter as the response variable. We included population, age, and sex as additional fixed effects and individual bird as a random intercept. We ran separate models for spring and autumn migration periods and their respective adjacent summer and winter periods (8 models total).

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SUPPLEMENTARY FIGURE CAPTIONS

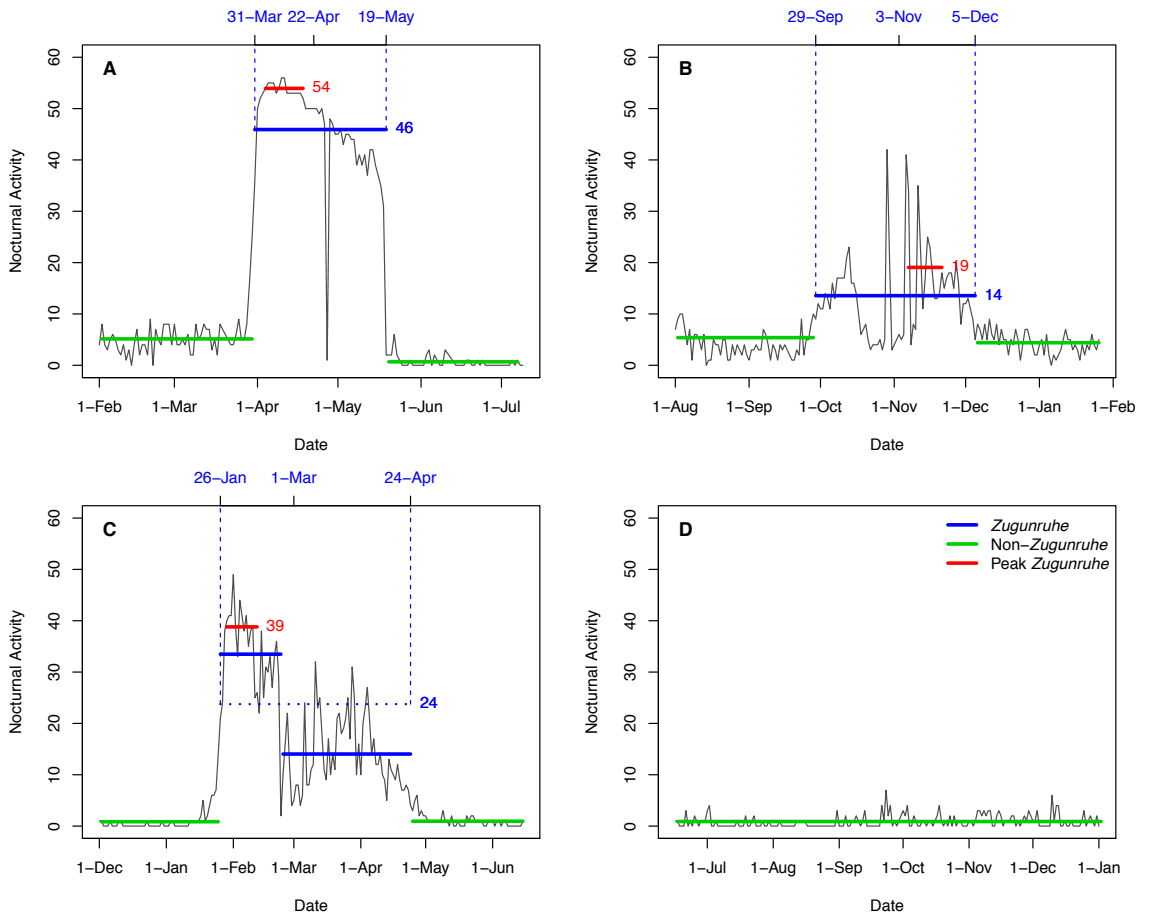


Figure A1. Example data analysed with changepoint analysis. The solid black line shows an individual bird's pattern of nocturnal activity, quantified as the number of active ten-minute periods in a night. The coloured lines show the result of the analysis: solid blue lines show elevated time segments classified as *Zugunruhe*, and solid green lines are those not classified as *Zugunruhe*. Blue dates on top of each figure show the onset, mean, and end of the *Zugunruhe* time segments. The solid red line shows the 15-day period with the highest level of activity. When changepoint analysis identified more than one elevated *Zugunruhe* segment but the segments were adjacent (see C), mean *Zugunruhe* intensity was the mean activity level across all elevated segments (blue dotted line). (A) Spring data from a Siberian stonechat. (B) Autumn data from an Austrian stonechat. (C)

Spring data from an Irish stonechat. **(D)** Autumn data from an Irish stonechat, an individual that did not exhibit *Zugunruhe* for that season.

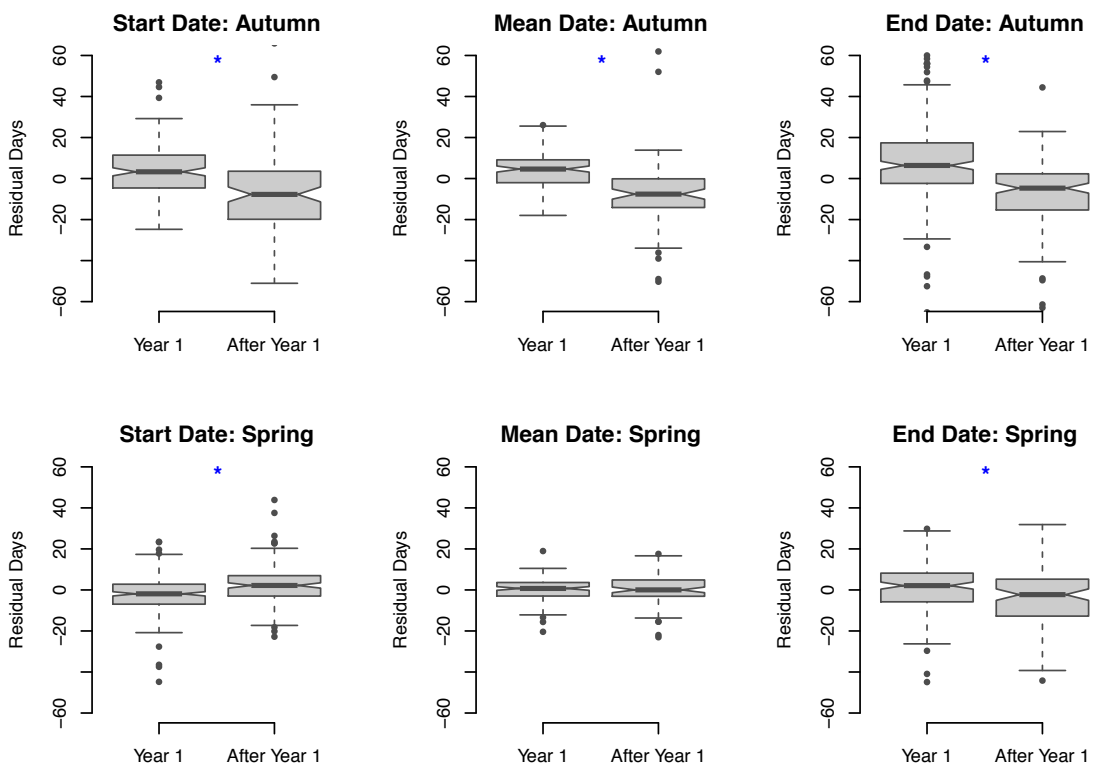


Figure A2. Age effects on timing of *Zugunruhe*. Boxplots show residuals from models on the timing of onset, mean, and end of *Zugunruhe* plotted against bird age group (first year or older) for all populations combined. Top row: autumn migration period; bottom row: spring migration period. A blue asterisk indicates significant differences of first year compared to older birds. Y-axes are standardized and some outliers are cut off in order to better visualize differences.

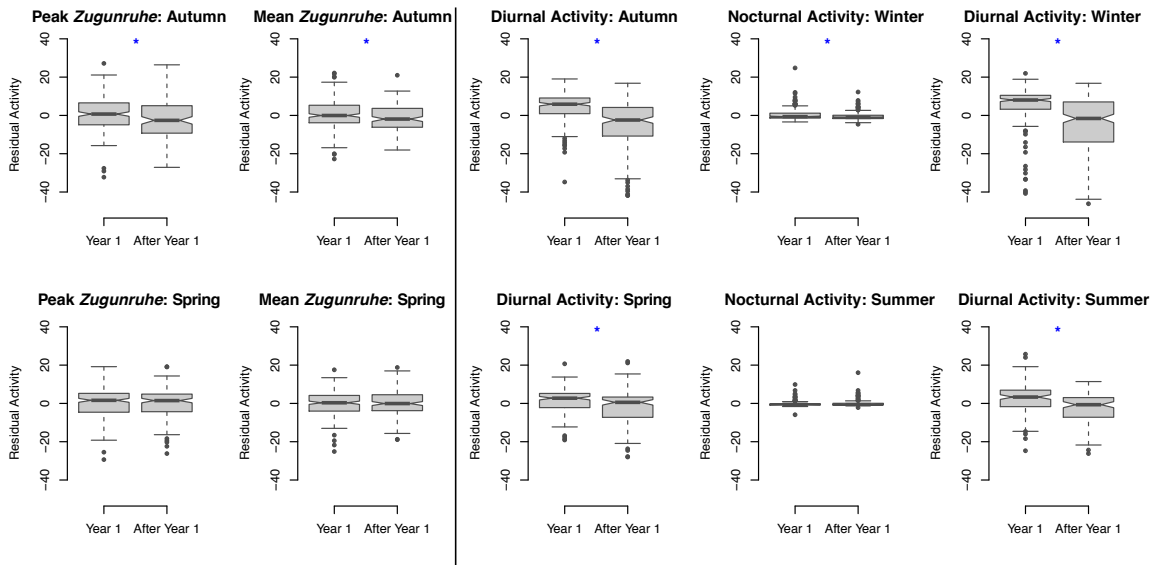


Figure A3. Age effects on diurnal and nocturnal activity levels. Boxplots show residuals from models on activity levels plotted against age group (first year or older). Diurnal activity is shown for all four seasons. Nocturnal activity is shown for all birds in summer and winter, but during migration seasons, only for birds that are identified as migrants using changepoint analysis. Left panel: data on two measures of *Zugunruhe* level (peak and mean) for autumn (top) and spring (bottom). Right panel: activity data for all birds during autumn and spring (diurnal only), and during winter and summer (diurnal and nocturnal). A blue asterisk indicates significant differences between the age groups.

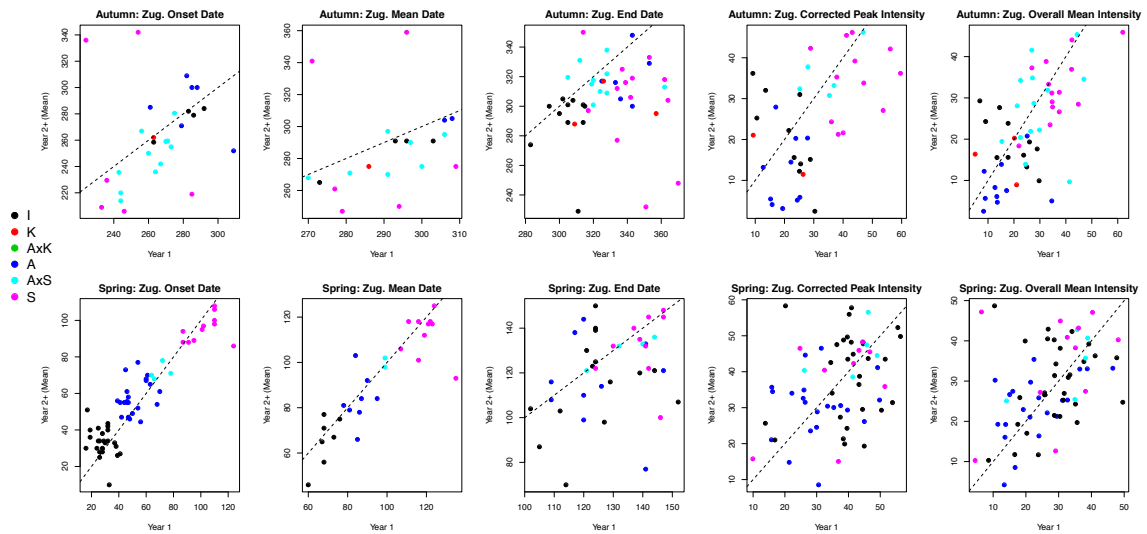


Figure A4. Relationship of *Zugunruhe* metrics during a bird's first year with those of later years, stratified by population. The figure shows the value of a given *Zugunruhe* metric in year 1 of a bird's life (x-axis) and the mean of all subsequent values of this metric in later years of its life (y-axis). Top panel: autumn, bottom panel: spring. Colour coding indicates the different populations. The dotted line is the identity line. Spring onset and mean date show the strongest relationship, but one that is driven entirely by inter-population differences (see Figure A5). However, sample sizes are small and some populations are not represented because many birds were not tested for successive years, or did not engage in *Zugunruhe* in one or more years.

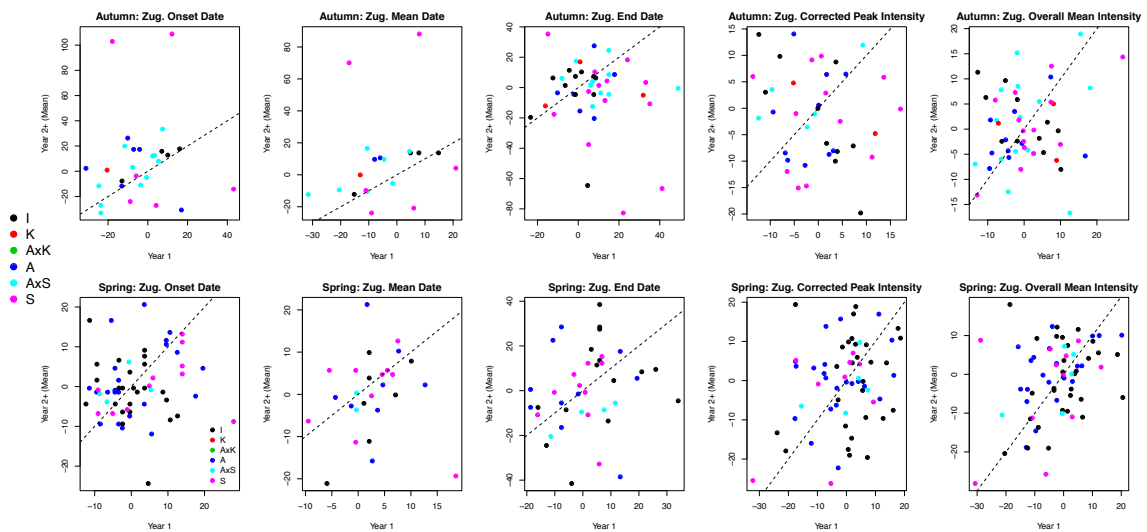


Figure A5. Relationship of *Zugunruhe* metrics during a bird's first year to those of later years (see Figure A4), but standardized to respective population means. After accounting for variation explained by population means, formerly strong relationships (see Figure A4) became non-significant. Here, only three comparisons show significant relationships (not corrected for multiple comparisons): spring corrected peak ($P = 0.028$), spring overall mean ($P = 0.003$), and fall overall mean ($P = 0.049$). Data plotted as in Figure A4.

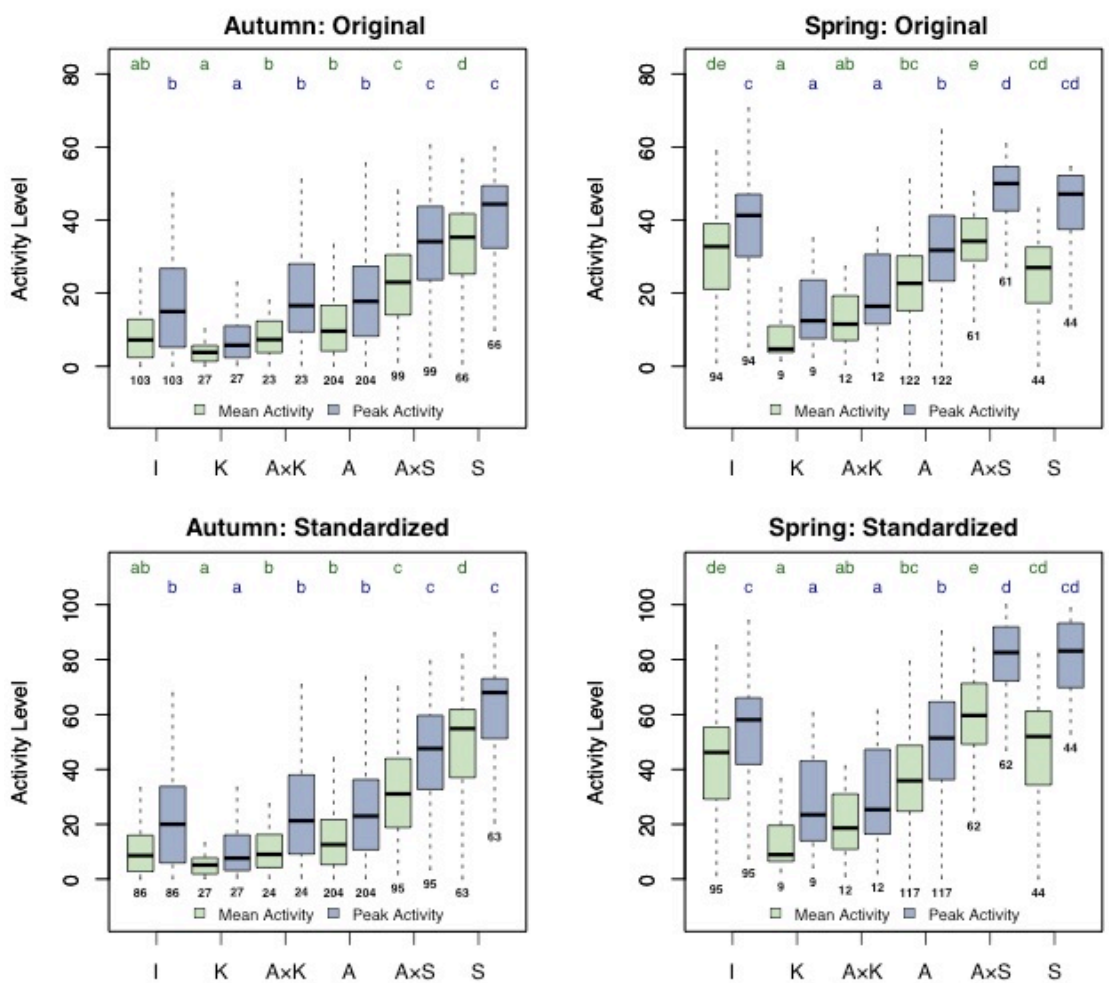


Figure A6. Nocturnal activity levels during the autumn and spring migration seasons, comparing mean and peak activity, and visualising the effects of accounting for night length. “Mean activity” is the average nocturnal activity level of a bird during the most active 60-day period for its population. “Peak activity” is the average nocturnal activity level of a bird during its most active 15-day window of the 60-day period. Top row shows boxplots of the raw (original) data; bottom row shows the result after representing activity as a proportion of night length. In the bottom row, a value of 100 indicates a bird was active for the entire night. Letters shown at the top of plots indicate significant pairwise differences: groups that do not share a letter are significantly different. Most results are similar between the top and bottom rows; the notable exception is that Siberian stonechats, and to a lesser extent Austrian \times Siberian stonechats, show a higher peak

activity level when expressed as a proportion of night length. This is because Siberian stonechats begin *Zugunruhe* relatively late in spring when nights are shorter than during their autumn migration. Hence, as a proportion of night length, Siberian birds are almost continuously active during spring.

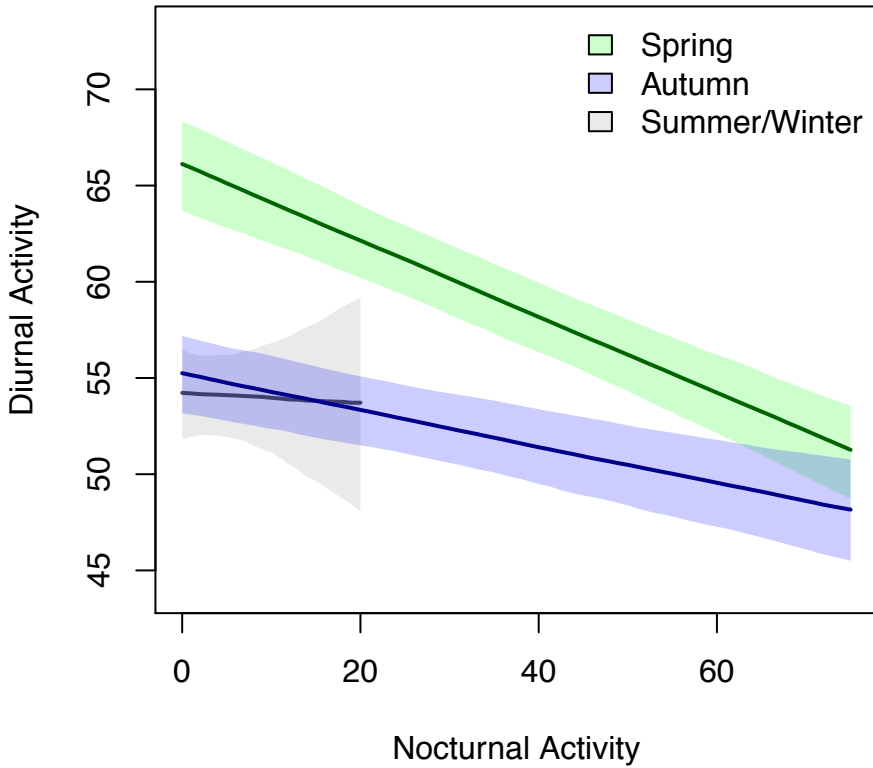


Figure A7. Daily association between nocturnal and diurnal activity during spring and autumn *Zugunruhe* periods and during summer and winter (combined). Plotted are model predictions and bootstrap 95% confidence intervals calculated with the *bootMer* function in the R package *lme4* (Bates et al. 2015). Nocturnal activity covaried negatively with diurnal activity during spring and autumn, but not in summer and winter. For summer and winter, there was a significant effect of sex on diurnal activity; predictions shown are for males only.