Costs of migration
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Consequences of avian migration on subsequent reproduction? An experimental approach in Rose Coloured Starlings

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ABSTRACT

Many temperate zone bird species are migrants and have to invest much energy into flight to reach their summer and winter quarters. Many studies have shown how migration affects body physiology, including the accumulation of energy stores and the reduction of non-essential organs. In spring, the costs of migration may trade-off with preparations for breeding, such as the timing and extent of the development of primary and secondary sexual traits. It has been shown that birds arriving early in the breeding grounds have a higher reproductive success than late birds, but no study to date has addressed whether and how migration itself influences reproduction. Using a wind tunnel, we investigated the effect of the high workload during long flights on body condition and on successive reproduction. We let an experimental group of male Rose Coloured Starlings (*Sturnus roseus*) fly in the wind tunnel every day and cover a total flight distance of more than 4700 km in 49 days. They were compared with a control group of males that were not flown. After "migration" individuals from both groups were kept in a common breeding aviary, where they directly competed for nest boxes and females. The increased workload during "migration" did not significantly affect any of the spontaneous seasonal changes in fat score, in breast muscle thickness, in plasma testosterone levels, and in bill and mantle colour compared to the control group. Body mass increased more slowly in experimental than in control birds, but it reached the same level soon after "migration". We did not observe any effect of the experimentally increased heavy workload on behaviour during the early breeding phase or on any parameter of reproductive success. We thus failed to find a trade-off between long flight and the development of traits in preparation for breeding or reproductive success. A possible treatment effect might have been obscured by the unrestricted food supply. We discuss the alternative of effects on other life-history stages such as future survival, migration or reproduction. The data in any case attest to the strong endogenous control of seasonal physiological changes in preparation for breeding that occur independently of the extreme effort invested in long distance migration.
INTRODUCTION

For many birds life is particularly taxing during spring. Migratory birds have to invest much energy into flight during long-distance migration. This investment may trade-off with investment in reproduction immediately thereafter. The flight costs themselves are also subject to trade-offs, which have been modelled in optimal migration theories. Birds are expected to either minimise the energy or the time spent during flight (Alerstam and Lindström 1990, Lindström and Alerstam 1992, Hedenström and Alerstam 1997). Flying more economically may result in later arrival, but this also comes at a cost. Earlier arriving birds have a larger choice of breeding sites, territories, and mates (Marra et al. 1998), and a broader time window for recovery from migration and for successive reproduction. Birds arriving earlier at the breeding grounds often occupy better territories and gain a higher pairing and fledging success (Alatalo et al. 1986, Lozano et al. 1996, Hasselquist 1998, Naef-Daenzer et al. 2001). Migratory flights are demanding, and several studies have tracked changes in physiology and body composition during that time. On the one hand, body mass and breast muscle (M. pectoralis) size decrease during long flights (Lindström et al. 2000, Swaddle and Biewener 2000, chapter 3), and several organs, except for lungs and brain (Battley et al. 2000), can be reduced during migration (Hume and Biebach 1996, Piersma and Lindström 1997, Biebach 1998). On the other hand, gonadal development starts already during spring migration in preparation for the following breeding period (Bauchinger 2002, Bauchinger et al. 2005, Ramenofsky and Wingfield 2006).

We may expect the high costs of migratory flight to trade-off with investment in breeding, but whether the high workload associated with prolonged flight affects subsequent reproduction has hardly been investigated. A study on Zebra Finches (Taeniopygia guttata), investigating the effect of hopping activity on reproduction, showed that reproduction was delayed in birds with a higher workload, without an effect on clutch size (Deerenberg and Overkamp 1999). Another study on Zebra Finches also observed delayed reproduction in birds which had experienced reduced food intake rate and a higher DEE during six weeks before reproduction, but were kept under ad lib food conditions during breeding (Wiersma and Verhulst 2005). In these two studies, not only workload was manipulated, but also food was restricted at the same time. Here, we decoupled the effects of an increased workload and caloric restrictions.

To understand how birds trade-off costs of flight, preparation for reproduction, and timing of arrival, one needs to know the cost of migration in terms of reduced condition during and at the end of migration and of breeding
performance. The aim of this study was to experimentally investigate the effect of a high workload associated with prolonged flight on an individual's condition and on its subsequent reproductive performance when fed ad libitum, i.e. decoupled from effects of food supply. We used the migratory Rose Coloured Starling as a model species, because the birds easily breed in captivity and quickly learn to fly in a wind tunnel (Engel et al. 2006). During the spring migratory season, we let an experimental group of males fly about 4700 km each in a wind tunnel and kept a second group of males under the same environmental and feeding conditions without the flights (control group). During the "migration" period, we measured the daily energy expenditure with the doubly labelled water method (Speakman 1997). We also recorded changes in body mass, fat store, pectoralis size, levels of circulating testosterone, and bill and plumage colouration. After the "migration" period, all birds of both groups were brought into a large aviary such that they could directly compete for nest sites and females. Under the hypothesis that prolonged flight during migration is costly and that it trades off with investment in breeding, we predicted that the experimental group would show (1) a larger decrease in body mass and pectoral muscle size (Lindström et al. 2000, Swaddle and Biewener 2000, chapter 3); (2) delayed development of the gonads, resulting in lower plasma testosterone levels; (3) less intense colouration especially of the bill (presumably a sexual trait); (4) delayed and less successful reproduction compared to the control group (Deerenberg and Overkamp 1999, Wiersma and Verhulst 2005).

MATERIALS AND METHODS

Birds

Rose Coloured Starlings are migratory birds with their breeding range stretching from Central Asia to Eastern Europe and their wintering grounds from Pakistan to south India (Hudde 1993). Birds live and migrate in flocks and breed in colonies. They build their nests in cliffs, under roofs or in stone walls (Schenk 1929, Augustijn 1997, Miltchev and Tschobanov 2002). In the Ukraine, we observed that males arrived earlier in the breeding area than females, defended one or more nesting sites and guarded their mate. Plumage colouration became more intense "rose" coloured (personal observations), and the pale pink and brown bill colour changes to a more intense magenta and blueish-black during spring (Hudde 1993). As male Rose Coloured Starlings present their back when dancing around females during courtship, we propose that the colouration of the
mantle feathers might be used as a sexual signal. Bill colouration might be used as signals for both males and females.

The birds in this experiment originated from a breeding colony on the Crimea peninsula, Ukraine. In 2001, nestlings were taken at the age of 7 days, and hand-raised at our institute in Seewiesen, Germany. The birds thus were used to handling from the beginning and had already learned to fly in the wind tunnel. All birds used in the experiment were experienced breeders. We randomly assigned eight males to the experimental group, which "migrated" in the wind tunnel, and eight males to the control group.

"MIGRATION" PERIOD

Housing

The 16 males were kept in groups of four in aviaries (ca. 2 m x 2 m x 2 m) adjacent to the wind tunnel, with birds of the experimental and the control group separated. Each aviary was equipped with three perches and two feeding trays. The starlings were fed standard food (dried insects, heart, rusk, curd, and egg, supplemented with minerals and vitamins), Realpasto®, and fresh fruit ad libitum, and had unlimited access to fresh water. We used Osram Biolux® lamps simulating the frequencies of natural daylight. Day length was adjusted weekly according to the photoperiodic conditions the birds would experience during spring migration in the field, without accounting for the shift of photoperiod caused by the east-west migration. At the beginning of simulated migration on March 1st, LD was 12.4:11.6 (12.4 h from dawn civil twilight to dusk civil twilight) equivalent to northern India (27.5°N). By the end of "migration" on April 18th, day length had increased to LD 14.6:9.4, corresponding to the conditions in southern Ukraine (47.5°N, Figure 5.1 A).

Wind tunnel and "migration"

Experimental birds were flown under controlled conditions in our wind tunnel in Seewiesen (for a detailed description, see Engel et al. 2006 and chapter 1) in groups of four. Experimental birds flew in the wind tunnel during a total period of seven weeks from March 1st until April 18th, 2004. During the first week, all experimental birds flew only short flights of 0.25 - 1.5 h (0.6 ± 0.8 h per day) to get used to the wind tunnel (Figure 5.1 B). From the second week onwards, flight time was on average 3 h per working day and 1 h each day during the
Figure 5.1
Day length (A), cumulative distance covered (B) and sampling schedule during the "migration" period (C). Measurements were taken of daily energy expenditure (C1), colouration (C2), plasma testosterone (C3), and body mass, fat score, and relative breast muscle thickness (C4).
weekends. Over the entire 7-week period, daily flight time was on average 2.2 ± 1.2 h per bird. During that period, each bird of the experimental group flew more than 109 h in total. With the flight speed set to 12 m s\(^{-1}\), the experimental birds thus covered a total distance of more than 4700 km (Figure 5.1 B). This is equivalent to the distance birds would migrate between northern India and the Crimea, Ukraine. Birds of the control group experienced the exact same conditions as experimental birds (like temperature, day length, level of human presence), except that they did not fly in the wind tunnel. The control birds were not prevented from locomotory activities like hopping or short flights within the aviaries.

**Measurements**

During the "migration" period, physiological and morphological variables were measured for all birds according to the schedule indicated in Figure 5.1.C.

*Daily energy expenditure (DEE) and body composition*

We measured the energy expenditure of experimental and control birds during 24 h (DEE) on March 31\(^{st}\), halfway through the "migration" period. Birds of the experimental group flew for 3 h in the wind tunnel during the measurement.

In the morning, we injected all 16 birds intraperitoneally with 0.22 g doubly labelled water (DLW), weighed on an analytical balance (Sartorius BP 1215) to the nearest 0.1 mg. Isotope enrichments for \(^{18}\)O and \(^{2}\)H were 60.4 and 36.5 atom percent, respectively. To let the DLW evenly equilibrate with the body water, each bird was kept in a dark box without access to food or water. After one hour, an initial blood sample of about 60 µl was taken from the jugular or brachial vein. The bird was weighed to the nearest 0.01 g ("initial body mass") and brought back to the aviary, with access to food and water. We started the flights at least 2 h after the initial blood sample was taken, to minimise effects of handling and blood sampling on flight performance. The final blood sample of 60 µl was taken 24 h after the first blood sample. Afterwards, birds were weighed again to the nearest 0.01 g ("final body mass"). We calculated the average body mass as the mean between initial and final body mass. From uninjected Rose Coloured Starlings, which were not used for this experiment but kept under corresponding conditions, we collected blood samples to assess the background levels of \(^{2}\)H and \(^{18}\)O. All blood samples were subdivided over four capillaries, immediately flame-sealed, and stored at 8°C for further analysis in
the Centre for Isotope Research, Groningen, The Netherlands. Analyses were done in duplicate for the initial and in triplicate for the final blood samples. For analytical procedures employed see chapter 1 or Engel et al. (2006). The rate of carbon dioxide production was calculated using equation 7.17 from Speakman (1997), which was converted to the level of energy expenditure by using an energy equivalent of 27.3 kJ l⁻¹ CO₂ based on protein-rich diet (Gessaman and Nagy 1988a).

We calculated the water efflux rates (rH₂Oout, in g day⁻¹) during the 24 h measurement period using Nagy and Costa's (1980) equation 4, assuming the proportion of the water flux lost by evaporation was 0.25 (Speakman 1997) and a fractionation factor of 0.94 as recommended by Speakman (1997). Water influx rates (rH₂Oin, in g day⁻¹) were assessed from the change in total body water (TBW) during the measurement and the fractionation-adjusted water efflux rate rH₂Oout. We calculated the volume of TBW based on the difference in ¹⁸O concentrations of the background and the initial samples after equilibration, correcting for an overestimation of 1.8% (Speakman et al. 2001). Based on the TBW measurements, we then calculated the mass of lean wet tissue (which is considered to contain about 73% water, Speakman 2001) and the fat content. To account for differences in body size, data on TBW and water flux are given relative to body mass (TBW₉₅ in %, and rH₂O in g day⁻¹ kg⁻¹).

**Body measurements**

Body mass, fat score, and relative breast muscle thickness were recorded between 9:30 and 11:00 CET on a weekly basis (Figure 5.1 C). Body mass was measured to the nearest 0.01 g with a Sartorius BL 1500 S balance. Fat scores were visually assessed according to Kaiser (1993) with the fat scores for furcula and abdomen averaged. The height of the breast muscle was measured relative to the sternum (for details, see chapter 3). We measured the relative breast muscle thickness in threefold and averaged these measurements for further analysis.

Body measurements were also recorded after the "migration" period on April 20th, immediately before the birds were moved to the breeding aviary.
Plasma Testosterone

Plasma testosterone levels were measured as an indicator of reproductive state at the beginning, during, and after the experimental treatment (Figure 5.1 C). Blood samples of 200 µl were collected with a syringe from the jugular vein and rinsed through heparinised capillaries to prevent agglutination. After centrifugation (10 min at 14000 rpm), the plasma was collected and stored first at -21°C and then at -70°C until analysis.

Testosterone concentrations in the plasma samples were measured with a radioimmunoassay following the procedure of Goymann et al. (2006). Antiserum against testosterone was obtained from Esoterix Endocrinology (Calabasas Hills, USA; No. 73-125), standard steroids were purchased from Sigma-Aldrich (Munich, Germany) and labelled steroids from Perkin Elmer (Rodgau, Germany). All chemicals used were of analytical grade.

Aliquots of plasma (15 µl - 100 µl) were brought up to 300 µl with double-distilled H₂O and equilibrated overnight at 4°C with ~1500 dpm of ³H-testosterone (Perkin Elmer NET-553) to estimate hormone recovery. Afterwards, 4 ml dichloromethane were added. After 4 h of equilibration the organic phase was separated from the aqueous phase by freeze-decanting. After a second extraction with 2 ml of dichloromethane the samples were dried under a stream of nitrogen gas at 39°C. The extracts were resuspended in 300 µl phosphate buffered saline with 1% gelatine (PBSG) and left overnight at 4°C to equilibrate. Aliquots (80 µl) of each fraction and sample were transferred to scintillation vials, mixed with 4 ml scintillation fluid (Packard Ultima Gold) and counted to an accuracy of 2 - 3% in a Beckman LS 6000 β-counter to estimate individual extraction recoveries, which were 89.4 ± 1.1% (average ± 95% CI). The remainder was stored at -40°C until RIA was conducted.

Standard curves were set up by serial dilution of stock standard solutions with a concentration range of 0.4 - 200 pg of testosterone (in duplicate). Dilutions of the antiserum (1/200) were added to the standard curve, to controls and to duplicates of the sample fractions (2 x 100 µl). After 30 min, 13500 dpm of ³H-testosterone were added and samples incubated for 20 h at 4°C. Antibody-bound and free fractions of testosterone were separated at 4°C by adding 0.5 ml dextrancoated charcoal. After 14 min incubation with charcoal the samples were spun (3600 g, 10 min, 4°C) and supernatants decanted into scintillation vials at 4°C. After adding the scintillation liquid, the vials were left to equilibrate for at least 4 h and then counted. Standard curves and sample concentrations for the assay were calculated with Immunofit 3.0 (Beckman Inc., Fullerton, CA, USA).
All samples were analysed within one assay. The intra-assay coefficient of variation was 11.1%, the lower detection limit was 0.7 pg per tube. Data were log-transformed for the statistical analysis.

**Plumage and bill colour**

Plumage and bill reflectance were measured with a spectrometer at the beginning and at the end of the experimental treatment (Figure 5.1 C). We recorded the reflectance spectra of the bill and of the feathers of the mantle and the rump from 300 to 700 nm which encompasses the visual sensitivity of passerine birds (Hart 2001). We used an Avaspec 2048 spectroradiometer and an Avalight-DHS Deuterium Halogen light source (Avantes, Eerbeek, The Netherlands), connected through a bifurcated fibre optic probe. A cylindrical plastic tube was mounted on the optic probe to exclude ambient light and to standardise the measuring distance. Each sample was measured five times with the probe held perpendicular to the sample. For bill, we measured the pink area close to the nostril, three times on the left side, two times on the right. The feathers on the rump and mantle had a black rim, but we measured the pinkish part only. The reflectance was calculated relative to a white standard (WS-2) with the software Spectrawin (Top Sensor Systems). The five spectra for each sample were averaged and mean reflectance was summarised over 4.4 nm steps (“binned”; Grill and Rush 2000) for further analysis. The reflectance spectra of bill, mantle, and rump were then analysed using principal components (PCA; see Cuthill et al. 1999). The first three principal components explained most of the observed variance (bill: 99.1%, mantle: 97.3%; rump: 98.1%), and were used in t-tests as described below. PC1 explained most of the variability in reflectance (bill: 94.6%, mantle: 54.7%, rump: 80.8%) and is usually interpreted as achromatic brightness (Cuthill et al. 1999). PC2 explaining 2.9% of the variability in the bill and 33.5% in the mantle spectra corresponded to high reflectance in UV and red. For the rump analysis, it explained 14.5% and corresponded to high reflectance of intermediate wavelengths. PC 3 corresponded to high reflectance in UV in all analyses, explaining 1.5%, 9.0%, and 2.8% of the observed variability in reflectance for bill, mantle, and rump, respectively.

We took digital pictures from the bill with a Canon Eos 10 D camera with a circular flash (MR 14EX) under standardised conditions at the beginning and at the end of the experimental treatment. The distance between the camera and the bill (69 cm), focal widths (F 22), exposure (1/200 s), and grey background
were constant. White balance was adjusted in the program "Capture One". Pictures were analysed with Photoshop to determine hue, saturation, and brightness (HSB) of the pink area in the front of the left nostril. Each picture was measured 5 times. Hue is on a degree° scale, and our measurements lie between 340° to 5° (360° is red; Kuehni 1999, Harold 2001). We linearised the scale by setting 360° to 0° and calculating values between 340° and 359° as negative (e.g. 356° became - 4°).

BREEDING PERIOD

The breeding aviary

All males from both treatment groups (N= 15, one experimental bird was excluded because of an injured leg) were transferred to a breeding aviary at 12:00 on April 20th (henceforth referred to as day 0). Immediately afterwards, an equal number of females (N= 15) was released into the aviary.

The breeding aviary was 16.6 m long, 3.2 m wide, and 2.6 m high and was semi-open to let in daylight. The birds were kept under natural local photoperiodic conditions (47.5°N), corresponding to those at their natural breeding grounds in the Ukraine. The aviary contained 8 equal sections (2.1 m x 3.2 m x 2.6 m, l x w x h), each with an artificial wall with two nest boxes (0.21 m x 0.25 m x 0.21 m, l x w x h, with entrance hole diameter 5.3 cm). The nest boxes were fixed behind a brick wall to resemble natural breeding sites (Figure 5.2). In each section, we provided straw, hay, fresh turf-sward, and coconut fibres as nesting material. We also supplied each section daily with the same amount of standard food (see above), fresh fruit and salad, and with fresh water. Thus, resources such as nest sites, food, and nesting material were distributed equally over the aviary. Birds began inspecting nest boxes on the day they had been moved into the aviary, and they started nest building on day 2.

Behavioural observations

We started the observations 75 min after the birds had been transferred to the breeding aviary. Each section was observed for 15 min, and again for 15 min later in the afternoon on day 0. Two people simultaneously observed the birds' behaviour at two sections. From day 1 to 3, each section was observed for 1 h (30 min starting at 08:00 h, 15 min starting at 10:30, and 15 min starting at 15:00). On day 4 and 5, each section was observed for 30 min, starting at 8:00.
In total, each individual was observed during day 0 - 5 on average for $2.2 \pm 0.6$ h (range: 1.3 - 3.3 h).

We recorded the duration of singing and of other courtship displays (such as crest-raising, tail-fanning and wing-waving or flapping, or presenting the back while singing, "dancing" around the female, or presentation of nesting material), and the frequency of and the performance in aggressive behaviours between males (threat display, chasing, physical fights). For the analyses, the duration of observed behaviours is expressed as the proportion of the total time a particular bird had been observed. For each male involved in an aggressive interaction, we also calculated the proportion of interactions won, \textit{i.e.} the proportion of chasing or threatening other males or winning physical encounters. Proportions were arcsine-transformed before analysis.

\textbf{Recording of reproduction}

Every second day after the morning behavioural observations we checked the nest boxes and recorded nest building, the number of eggs laid, and hatching. We do not have information about extra-pair paternity, hence estimates of male reproductive success refer to within-pair success only. The experiment was stopped after the first chick had hatched, \textit{i.e.} clutches initiated after day 48 were not considered here.

\textbf{Figure 5.2}

Breeding aviary with eight sections. Each brick wall provided two nest boxes, their openings are indicated by arrows.
Statistical analyses

All analyses were done in SPSS 14.0, using two-tailed tests. Data shown are average ± SD. We used t-tests with separate variance estimates to analyse differences between the control and experimental group and paired t-tests to analyse within-individual changes over time. We applied Restricted Maximum Likelihood (REML) mixed models to test for changes over time if a parameter was measured at least three times during the "migration" period. Individual was entered in the model as a random factor, and time, group and group*time were introduced as fixed factors. Models were simplified in a stepwise manner, starting with the exclusion of non-significant (p> 0.10) interactions.

RESULTS

"MIGRATION" PERIOD

Daily energy expenditure, DEE

DEE was on average 55% higher in experimental than in control birds (experimental (N= 8): 224.4 ± 21.0 kJ d⁻¹; control (N= 8): 144.5 ± 25.0 kJ d⁻¹; t= 6.94, p< 0.001). Correspondingly, both water influx (rH₂Oᵢn; experimental: 24.1 ± 3.7 g d⁻¹, control: 16.8 ± 3.4 g d⁻¹) and water efflux (rH₂Oᵢₜ; experimental: 24.6 ± 3.5 g d⁻¹, control: 17.6 ± 3.1 g d⁻¹) were about 40% higher in experimental than in control birds (rH₂Oᵢn: t= 4.14, p< 0.01; rH₂Oᵢₜ: t= 4.28, p< 0.01). During the DEE measurements, body mass did not significantly differ between the two groups (experimental: 82.5 ± 9.1 g, control: 88.4 ± 11.1 g; t= 1.16, p= 0.27).

Body condition

During the first week of "migration", when birds were getting used to the daily handling routine, body mass decreased significantly (paired t= 4.28, N= 16, p< 0.01, Figure 5.3 A). This effect was similar for experimental and control birds (t= 0.61, df= 13.56, p= 0.55). Then, between March 9th and April 13th, body mass increased significantly (Figure 5.3 A, REML mixed model; time: F 5,40= 16.41, p< 0.001). As predicted, this increase appeared to be smaller in experimental than in control birds (Figure 5.3 A; group: F 1,14= 0.63, p= 0.44; group*time: F 5,40= 2.45, p= 0.05). As a consequence, body mass was lower in experimental than in control birds between March 16th and April 13th, when experimental birds
experienced the high daily workload (group: \( F_{1,71} = 4.63, p < 0.05 \); time: \( F_{4,28} = 2.54, p = 0.06 \)).

Similarly, fat scores increased significantly from March 9th onwards (Figure 5.3 B; REML mixed model; time: \( F_{5,53} = 4.66, p < 0.01 \)) with experimental birds showing significantly lower fat scores (group: \( F_{1,18.2} = 6.21, p < 0.05 \); the non-significant interaction group*time was excluded from the model).

Relative breast muscle thickness also increased significantly from March 9th onwards (Figure 5.3 C; REML mixed model; time: \( F_{5,45} = 6.53, p < 0.001 \)) but we did not observe a difference between the two groups (group: \( F_{1,18} = 0.62, p = 0.44 \); group*time: excluded from the model).

Total body water in relation to body mass (TBW \%) was higher in experimental (59.3 ± 5.1\%) than in control birds (52.8 ± 4.5\%; \( t = 2.69, df = 13.79, p < 0.05 \)) on March 31st. Experimental birds therefore had a higher lean wet mass (66.8 ± 3.05 g) and a lower amount of fat (16.1 ± 7.8 g) than control birds (lean wet mass: 63.9 ± 3.1 g; fat: 25.3 ± 8.9 g).

**Plasma testosterone**

Plasma testosterone levels increased during the "migration" period, but without a significant difference between the two treatments (Figure 5.4; REML mixed model with log-transformed data; time: \( F_{2,44} = 80.78, p < 0.001 \); group: \( F_{1,44} = 2.31, p = 0.14 \)). At the end of the "migration" period, plasma testosterone levels were on average more than 70% higher in birds of the experimental than in those of the control group, but the difference was statistically not significant, due to large individual variation and small sample size (\( t = 1.20, df = 13.94, N = 16, p = 0.25 \)).

**Figure 5.3 (opposite page)**

Development of body mass (A), fat score (B), and relative breast muscle thickness (C) during the "migration" period. Averages (+/- SE) of the experimental group are depicted in black and of the control group in grey. Squares indicate measurements after the "migration" period.
### Diagrams

**A**
- **Y-axis:** Body mass [g]
- **X-axis:** Date
- Data points for different mass values are plotted over time.

**B**
- **Y-axis:** Fat score
- **X-axis:** Date
- Data points for different fat scores are plotted over time.

**C**
- **Y-axis:** Relative breast muscle thickness [mm]
- **X-axis:** Date
- Data points for different muscle thickness values are plotted over time.
Figure 5.4
Plasma testosterone levels at different days during and immediately after the "migration" period. Shown are averages ± SE (in pg ml⁻¹) of the experimental (black, N= 8) and the control group (grey, N= 8). Note the logarithmic scale.

**Colouration**

The reflectance spectra of the bill show a peak in the UV at around 370 nm and an increase in reflectance from 600 nm onwards (Figure 5.5 A). The principal component analysis of the reflectance spectra revealed a change of PC1 and PC2 with time (PC1: paired t= 2.27, p< 0.05, PC2: paired t= 6.02, p< 0.001, PC3: paired t= 1.10, p= 0.29; N= 16). This reflects a decrease in overall reflectance (PC1) and a relatively smaller decrease in the UV part of the spectrum and at longer wave lengths compared to other wave lengths (PC2). The change was independent of the treatment (Table 5.1).

The analysis of photographs of the bill revealed a significant decrease in hue, *i.e.* a shift towards a more purplish colour (paired t= 4.40, N= 16, p< 0.01), an increase in saturation, *i.e.* richer colours (paired t= -5.35, p< 0.01), and a decrease in brightness (paired t= 2.26, p< 0.05; Table 5.1). None of the changes differed between the two groups (Table 5.1).
The reflectance spectra of the mantle and the rump showed high reflectance in the UV and at long wavelengths with a trough in between (Figure 5.5 B and 5.5 C). Apart from an increase in the reflectance of the longer wavelengths in the mantle feathers, i.e. feathers appeared pinker at the end of the "migration" period, we did not detect a change in colouration, and there were no differences between the groups (PC analyses, data not shown).

Table 5.1
Bill colouration the beginning and at the end of the "migration" period. Shown are averages ± SD for the experimental (N= 8) and the control group (N= 8). Noted are also the results of the t-test for differences between the groups with regard to changes of PC1, of PC2, and of PC3, and in hue, in saturation and in brightness between March 3rd and April 15th.

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<tr>
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<td>27.7 ± 2.7</td>
<td>0.76</td>
</tr>
<tr>
<td>brightness</td>
<td>54.0 ± 4.0</td>
<td>56.6 ± 3.5</td>
<td>51.7 ± 2.7</td>
<td>53.5 ± 2.6</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Condition before arrival in the breeding aviary
On April 20th, when birds were transferred to the breeding aviary and two days after the last day of the "migration" period, the difference in body mass between the control and experimental birds had disappeared (Figure 5.3, t= 0.15, df= 11.45, N_{experimental}= 7, N_{control}= 8, p= 0.88). There was also no difference in fat score and relative breast muscle thickness (Figure 5.3; t= 1.65, df= 11.30, p= 0.13, and t= 0.59, df= 11.97, p=0.57, respectively).
BREEDING PERIOD

Behaviour during day 0 - 5

Song activity accounted for 17.5 ± 4.5% of the time budget of the males during day 0 to 5, and courtship behaviour for 13.4 ± 4.6% (Figure 5.7). The highest song activity compared to other behaviours was observed between day 1 and 3, while birds were engaged in other courtship activities mainly between day 3 and 5. There was no difference between the experimental and the control group in either song activity (t= 0.03, df= 11.91, p= 0.98) or in courtship behaviour (t= 0.13, df= 11.38, p= 0.90).

Similarly, neither the frequency of aggressive male-male interactions, nor the proportion of interactions won differed significantly between control and experimental birds (Figure 5.8; frequency: t= 1.78, df= 12.05, p= 0.09), interactions won: t= 0.73, df= 9.17, p= 0.49).

Reproductive success

In total, only eight of the fifteen males bred successfully (i.e. paired and produced at least one clutch), three experimental and five control birds. Two clutches were produced, for which no social male could be identified. One experimental and two control males were polygynous, i.e. they paired with two females simultaneously.

There was no obvious difference in the timing of pairing between the control and experimental birds. The first completed nests were observed on day 6.3 ± 2.89 in experimental and on day 7.8 ± 1.89 in control birds (N_{experimental}= 3, N_{control}= 5). Clutches were initiated on day 17.7 ± 3.21 in experimental and on day 20.6 ± 4.69 in control birds. Average clutch size was 8.0 ± 0.0 in experimental and 6.6 ± 0.82 in control birds. All eight males fed the chicks. Given the low sample sizes, no formal statistical tests were conducted.
Figure 5.7
The percentage of time males spent singing (A) and in courtship activity (B) 0 to 5 days after being transferred to the breeding aviary. Shown are mean + SE, with black bars referring to the experimental (N= 7) and grey bars to the control group (N= 8).
Figure 5.8

(A) The frequency of aggressive male-male interactions 0 to 5 days after the birds had been transferred to the breeding aviary. (B) The proportion of those interactions won. Shown are mean + SE. Black bars represent the experimental (N= 7), grey bars the control group (N= 8).
DISCUSSION

During the "migration" period, we observed seasonal changes of morphological and physiological parameters. In both groups, there was a strong increase in body mass, in fat stores, in relative breast muscle thickness, and in plasma testosterone levels. All birds showed a marked shift in colouration of bill and mantle feathers towards more pronounced UV and red. These changes were obviously endogenously triggered, probably as a direct consequence of increased day length (Gwinner 1973, 2003). We found only little evidence that the "migratory" flight affected body condition and no effects on future reproduction. During "migration", we observed a strong trend of a reduced increase of body mass in experimental than in control birds, a significantly lower body mass during the time of intensive "migration" (March 16th - April 13th), and significantly lower fat scores in experimental birds. The energetic costs of flight are positively correlated with body mass (see Schmidt-Wellenburg et al. in press, chapter 3), and body mass might have been kept low to save costs (Swaddle and Biewener 2000). Immediately after the "migration" period, experimental birds were able to reach the same body mass as the control group. Thus, by the time the birds were released into the breeding aviary, the two treatment groups did not significantly differ in any of the measured parameters. In the breeding aviary, the performance of both groups with regard to singing, courtship and aggression was indistinguishable. There were no consistent differences in reproductive success between the treatment groups. In conclusion, we observed seasonal changes in all parameters measured during "migration", but found little effect of the heavy labour of migration on condition and performance. We now compare our data with those of other studies that have manipulated workload to further discuss this surprising result.

Manipulation of the workload

During the DLW measurement during 24 h, each experimental bird flew for 3 h, corresponding to 130 km, and spent on average 80 kJ d⁻¹ or about 55% more than a control bird. Extrapolating the additional costs for flight to the entire duration of "migration" results in a total additional energetic cost of 2910 kJ for the 4700 km flight. This is as much energy as contained in 75 g fat and matches almost the total body mass of a Rose Coloured Starling at the beginning of the "migration". It also corresponds with the total amount of energy a control bird would spend during 20 days in the aviary.
Although experimental birds spent about 55% more energy during the "migration" period, they did not significantly differ from control birds at the end of migration except in body mass and fat score. We now discuss three potential explanations for a lack of an effect.

Were migrating birds able to compensate for the increased workload?

Flight costs for a Rose Coloured Starling of 82.5 g are estimated at 30.7 kJ h\(^{-1}\) (based on DLW measurements during prolonged flights in the wind tunnel, Engel et al. 2006) or 92.2 kJ during a 3 h flight. The daily energy expenditure measured was 79.9 kJ higher in the experimental than in the control group. The difference of 12.3 kJ may be attributable to the energy that control birds spent during 3 h of sitting in the aviaries (which would amount to 4.1 kJ h\(^{-1}\)). But if the measured DEE of the control birds is expressed in kJ h\(^{-1}\), we obtain a figure 6.0 kJ h\(^{-1}\). This value is already about 50% higher than the calculated 4.1 kJ h\(^{-1}\), even without taking into account reduced energy expenditure at night, which would yield a higher figure of energy expenditure during daytime than 6.0 kJ h\(^{-1}\).

In conclusion, the energy expenditure during non-flight must have been lower in experimental than in control birds. Birds of the experimental group might have been less active in the aviaries when they were not flying and/or they might have had a lower nocturnal energy expenditure, as observed in European Starlings (Sturnus vulgaris; Bautista et al. 1998) and Zebra Finches (Deerenberg et al. 1998). In the latter study, birds that were exposed to an increased workload (hopping between perches to obtain food) reduced their nocturnal energy expenditure to such an extent that their total DEE did not exceed that of control birds. In the European Starlings, birds had to work for food by walking or flying at either a high or a low intensity. Food intake in birds under the hard working regime was reduced, food utilisation increased, body mass reduced, overnight metabolism and in consequence DEE lower than in birds under the "easy" working regime (Bautista et al. 1998). This effect of nocturnal energy savings could not be confirmed in Great Tits (Parus major) in a field study (Wiersma and Tinbergen 2003): DEE and feeding rates were higher in females with an experimentally increased than in females with an experimentally reduced brood size. Nocturnal resting metabolic rate, however, was not changed by the treatment.
**Was the experimentally applied workload too low to affect the birds' condition?**

The experimental birds migrated for more than 4700 km, corresponding to the distance they cover in nature. Nonetheless, the flight schedule imposed on them may not match natural migration. Little is known about migration routes and migration patterns of Rose Coloured Starlings, and thus about the daily time spent flying or the duration of stopovers (Bezzel 1993b, Hudde 1993). Birds in the field probably fly for longer periods of time per day than we imposed, with stopover periods in between. These birds would not receive food *ad lib.* as in our experiment. It is likely that birds migrating in the field also have to spend more energy during non-flight than our birds. We have measured daily energy expenditure in free-living male Rose Coloured Starling on the Crimea peninsula with the DLW method during the early breeding phase in May - June 2003 (Schmidt-Wellenburg, unpublished). Compared to these birds (henceforth referred to as "Ukraine", N= 9), DEE in experimental birds was slightly but not significantly higher (Ukraine: 199.0 ± 29.9 kJ d⁻¹; t= 2.04, df= 14.31, p= 0.06). As the Ukraine birds were lighter (73.9 ± 2.6 g) than the experimental birds (t= 2.61, df= 8.01, p< 0.05), this slight difference in DEE disappeared completely when DEE was expressed in relation to body mass (Ukraine: 2.69 ± 0.37 kJ g⁻¹ d⁻¹; experimental: 2.74 ± 0.32 kJ g⁻¹ d⁻¹; t= 0.11, df= 15, p= 0.91). The early breeding period is demanding for males, because they spend most of their time with fights for nesting sites and females and in courtship displays. We assume, that DEE during migration in the field may be higher than during the early breeding phase, but so far there are no data available.

**Could birds compensate for the high workload on a short-term basis, but suffer long-term costs?**

It is possible that the costs of migration are not expressed in short-term fitness components such as mating success and clutch size, but would turn up if complete rate of gene propagation would be taken into account. Not much is known about long-term effects of high workloads. Kestrels (*Falco tinnunculus*) raising enlarged families with increased work rates and higher DEE died sooner than those raising reduces families (Daan *et al.* 1996). Two studies on Zebra Finches showed that reproduction was delayed in birds under a high workload compared to a lower workload (hopping for food), while there was no difference in clutch size (Deerenberg and Overkamp 1999, Wiersma and Verhulst 2005). Zebra Finches under a high workload also paid in terms of replacing experimentally removed feathers with shorter ones. These additional costs for
replacement of feathers added up to the high workload and resulted in a further delay of reproduction (Wiersma and Verhulst 2005). Several studies on chick development have shown carry-over effects into later life-history stages if chicks are reared under bad conditions. Some investigations showed that chicks reared in adverse conditions can catch up until fledging (Metcalfe and Monaghan 2001, Fitze et al. 2004). Although these birds do not necessarily suffer immediately from these bad circumstances during (early) development, they often pay later in life in terms of reduced survival, lower social rank, worse territory quality, smaller clutch size, and finally lower reproductive outcome (Gebhardt-Henrich and Richner 1998, Lindström 1999, Metcalfe and Monaghan 2001, Fitze et al. 2004).

Gustafsson and Pärt (1980) could show that clutch size in Collared Flycatchers (Ficedula albicollis) was affected by their reproductive effort earlier in life, which the authors interpreted as costly reproduction enhancing the rate of senescence. We cannot exclude that our experimental Rose Coloured Starlings similarly would pay later for the high workload during “migration”.

Condition during the "migration" period

The increase in body mass, breast muscle thickness and fat score during the "migration" period cannot be explained by food supply alone, as birds were kept under ad libitum conditions before the experiment as well. Apparently, these changes are endogenously programmed. The birds may store extra resources anticipating the high energy expenditure during migration, for the unpredictable situation at the breeding grounds, and for the high energetic costs during especially the early breeding phase (Bromley and Jarvis 1993). Just as the increase in body mass, also changes in plasma testosterone and colouration of bill and feathers appeared to follow a seasonal program. These endogenously triggered changes took place even in the absence of females or nesting sites, which might in the field act as additional stimuli.

Lindström et al. (2000) showed rapid changes in both muscle size and body mass in Red Knots (Calidris canutus) during fasting, flights of several hours in a wind tunnel, or refuelling. They hypothesised that changes in the pectoral muscle might be caused by protein metabolism, the need for a special protein:fat ratio, or by the regulation of flight capacity (i.e. a higher body mass requires a bigger muscle). Another study also observed a decrease in both body mass and pectoralis mass in exercising European Starlings (Swaddle and Biewener 2000). In our study, we observed an effect of exercise on body mass, but not on relative breast muscle thickness. Although we failed to measure an effect of "migration"
on relative breast muscle thickness, the measurements of TBW suggest that birds of the experimental group had more muscle tissue, as their lean wet mass was higher than in control birds.

**Breeding and reproduction**

We did not observe any differences between the experimental and control birds with regard to activity, behaviour, or performance in male-male interactions. Birds of both groups were ready to reproduce, and neither mating success nor timing of breeding appeared to be different. Two studies on Zebra Finches recorded a delayed reproduction after birds had been exposed to a high workload. Deerenberg and Overkamp (1999) exposed birds repeatedly to either a high or a low workload (hopping for food) during four weeks. These periods of manipulated workload alternated with five-week periods, when birds bred under *ad libitum* conditions. Clutch and brood size did not differ between the treatments. However, birds of the high workload started their clutches with a delay of 6 days. Another study kept birds under variable reward rates during six weeks, before the birds were allowed to breed (with unlimited access to food; Wiersma and Verhulst 2005). Zebra Finches of the high workload showed carry-over effects of these adverse conditions into the breeding period and started their clutches later. However, in both studies birds had to work (at lower or higher levels) for their food. Therefore, effects of an increased workload *per se* and of food restriction could not be separated.

**Conclusions**

We observed strong and probably endogenously programmed seasonal changes in body mass, relative breast muscle thickness, plasma testosterone levels, and colouration in Rose Coloured Starlings during the "migration" period. Most of these changes were not in any way affected by the heavy workload of flying over 4700 km in the wind tunnel, which increased energy expenditure by 55% in experimental compared to control birds. Changes in preparation for reproduction, such as plasma testosterone levels and colouration, occurred even in the absence of stimuli like females or nesting sites. It is attest to the strength of the endogenous program underlying annual migration that all the physiological changes preparing birds for breeding in an area far away from their wintering grounds actually take place at the same rates regardless of whether the birds are actually flying or not. The heavy workload during migration
obviously is also fully integrated in the seasonal organisation and exerts no discernable negative effect on subsequent reproduction.

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