Ea l Life E en Ca O e ● Infl ence P e-Mig a ● C●ndi i●n in a F ee-Li ing S●ngbi d

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1 D I B , G , G , , C , **2** D B , A F A R , , L , , C , **3** D B , B C , B , , A , **4** D B , C , , , A

Abstract

C - . I , . I , 13 1 (*Passerculus sandwichensis*). . H , - - , . - (—) - . . .

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nestling mass. We then examine the hypothesis that conditions during development affect 1st year survival through their effect on migratory body condition. Following this hypothesis, we predicted a positive relationship between pre-migratory lean tissue mass and pre-migratory fat mass with nestling mass. Using comprehensive experimental and observational data spanning multiple years and life-history stages, we provide the first evidence that conditions during development limit survival during migration through their effects on pre-migratory fat stores.

Methods

Fieldwork was conducted on Kent Island, an isolated 80 ha

correlation between deuterium dilution space and lean tissue mass as determined via chemical extraction ($R^2 = 0.84$) in a similar sized species to the Savannah sparrow, the black cap *S l ia d ica illa*. Second, any unexplained variation in mass not accounted for by total body water after accounting for skeletal size will be mostly due to fat.

For nestlings of altricial species, such as the Savannah sparrow, measurements of total body water using heavy water dilution may over estimate lean tissue mass for two reasons. First, deuterium may be incorporated into rapidly growing tissues, over estimating deuterium dilution space and total body water [44]. Second, the tissues of young nestling songbirds retain extra water [45]. However, the growth rate of both lean and dry mass in nestling Savannah sparrows reach their asymptotes by approximately 8 d of age [46] and excess tissue water in similarly sized rufous-winged sparrows *Aim hila ca ali* declines to adult levels by approximately 7 d of age [45]. Therefore, given the age of our study individuals during the pre-migratory period (≥27 d of age), heavy water dilution provides an accurate measure of lean tissue mass.

In 2008, 2009, and 2010, three, eight, and 22 individuals, respectively, were carrying radio transmitters during the pre-migratory period (0.62 g; Lotek, Newmarket, Ontario) as part of a larger study on migratory movements. In a recent analysis, we showed that radio transmitters had no effect on total body water or quantity of fat before migration [42]. Also, inclusion of a radio term in our models (0 = no radio, 1 = radio) was not significant and did not change the sign or magnitude of any parameter estimates in our final models (Table S5, Table S6, Table S7). Thus, for parsimony, this term was not included in our final models. Additionally, in 2008, all nestlings from 12 nests received phosphate-buffered saline as a nutritional supplement and all nestlings from four nests received a hypothesized immune system booster (lysozyme). Similarly, in 2009, one nestling from three nests also received phosphate buffered saline. However, neither treatment had an effect on nestling mass (Table S8) and removing these individuals had no effect on the relationship between clutch size and nestling mass in either our observational or experimental analyses (Table S9, Table S10), and so were not censored from any analysis.

Individuals were considered to have survived their first year if they were observed in any year following their natal year. Re-sighting probability within the study site was assumed to be close to 100% because observations of breeding behavior and nest monitoring ensured that individuals were sighted almost daily.

All analyses were conducted in R version 2.8.1 using the 'nlme' or the 'MASS' packages [47–49]. The effects of conditions during development on pre-migratory body condition were analyzed in a generalized multi-level path (GMLP) modeling framework using restricted maximum likelihood estimates [50,51]. A GMLP model is a type of path analysis that can easily accommodate small sample sizes, non-normally distributed data, non-linear functional relationships, and random effects [50,51]. The fit of a GMLP model is assessed using the concept of d-sep (distance separation) tests [50]. A d-sep test represents a test of the statistical independence between two variables. Shipley [50] shows that for each acyclic path model, there is a subset of independence tests referred to as a ''minimum basis set'' that account for all possible independence relationships. The null probabilities (P-values) from each test are used to calculate Fisher's C statistic: $C = -2\sum\ln(P)$), where P represents the null probability (the P-value) of each d-sep test (total = K), and where C follows a χ^2 distribution with 2*K degrees of freedom [50,51]. A significant P-value for this test ($P<0.05$), suggests the structure of the path model is not consistent with the empirical data. Once model fit is assessed, each path or structural equation is parameterized using the appropriate statistical model.

All model fitting was done using generalized linear mixed effects models. Significance of parameter estimates was evaluated at $\alpha = 0.05$. Significance of random effects was evaluated using 95% confidence intervals for the SD estimate of the intercepts. Prior to model fitting, potential curvilinear relationships were visually assessed using scatter plots fitted with loess lines. Model fit was visually assessed using residual plots. Correlations between predictors were evaluated through the variance-covariance matrix of the fitted model ($r\geq0.7$); in no case were model terms highly correlated. The approximate proportion of variation accounted for by each model was assessed via regressions of observed and fitted values derived from models without random effects. For the path analysis, each variable was standardized ($[value-\bar{X}]/SD$) such that path coefficients represent standardized partial regression coefficients, or the standard deviation change in y when x is increased or decreased by one SD [50].

Two-way interactions were initially assessed one at a time using the cross-product values of the terms involved in the interactions (Table S11). For those interactions that were significant, we replaced the cross-product terms with the standardized cross-product residuals according to Lance [52]. This procedure eliminates covariances between the interaction term and the terms involved in the interaction, thereby reducing the number of parameters (paths) in the path model. Only significant interactions were included in the final path model for parsimony.

B . We predicted that individuals from reduced broods would be in better condition at day 8 in the nest (see Introduction for hypotheses). We assumed that parental effort did not vary with clutch size [53]. Sample sizes were too small to experimentally assess the effect of number of nestlings on pre-migratory condition ($n<10$ recaptures of individuals from reduced treatments; Table S4). A random effect for natal nest was included to control for covariance among nest mates (Table S4).

A - : **1** . We predicted nestling mass would positively influence 1st year survival (see Introduction for hypotheses) and that the timing of nesting would negatively influence survival. We made the latter prediction because optimal conditions for migration are hypothesized to decrease through time, possibly limiting survival immediately before or during migration [54,55]. Random effects for natal nest nested within mother id nested within year were included to control for covariances among nest mates, related individuals, and individuals from the same cohort.

: . We predicted that nestling mass would be negatively influenced by the timing of nesting and brood size (Fig. 1; see Introduction for hypotheses). We controlled for year because of potential differences in resource availability, weather, and predators across years [31], [56]. We examined two-way interactions between timing of nesting and year with number of nestlings and nestling mass, as well as two-way interactions between clutch size with year, timing of nesting with nestling mass, and clutch size with nestling mass (Table S11). These interactions were examined because relationships between variables may vary across time owing to potential differences in environmental conditions both within and across years. Again, a random effect

for natal nest was included to control for covariances among nest mates. Only one randomly selected nest per adult female was included in the analysis.

. We predicted that nestling mass would be positively correlated with total body water

for a relationship between nestling mass with either timing of nesting ($\beta = -0.01$, $t = -1.0$, $df = 35$, $P = 0.321$) or year (2010: $\beta = 0.16$, $t = 0.51$, $df = 35$, $P = 0.616$).

A - : 1

First-year survival was positively related to nestling mass ($\beta = 0.13$, $t = 3.2$, $df = 1889$, $P = 0.001$, Fig. 3), negatively related to the timing of nesting ($\beta = -0.01$, $t = -2.3$, $df = 114$, $P = 0.021$), and positively related to tarsus length (linear term for tarsus length: $\beta = 2.27$, $\ell = 2.4$, $df = 1889$, $P = 0.018$; curvilinear term for tarsus length: $\beta = -0.06$, $\ell = -2.4$, $df = 1889$, $P = 0.017$). Regressing the

mass and timing of nesting ($\beta = -0.08$, $t = -0.9$, $df = 46$, $P = 0.399$).

Both total body water and fat mass varied with date captured,

better condition may also exclude individuals in poor condition from favorable foraging sites [25], [58], [76], again preventing individuals from compensating for their poor condition. Further studies linking conditions during development with body condition, behavior, and immune system function during the pre-migratory period are needed to adequately test these hypotheses.

Indirect effects in path models are calculated as the product of the path coefficients along the indirect path (Fig. 1). Our path model suggests that timing of nesting may affect pre-migratory body condition through two indirect mechanisms. First, timing of nesting had a negative indirect effect on pre-migratory fat mass through its effect on nestling mass ($-0.33 \times 0.24 = -0.08$). Second, the negative effect of timing of nesting on moult progression (see also [66], [70]), and the negative effect of moult progression on total body water (see also [59–62]), results in a positive indirect effect of timing of nesting on total body water ($-0.35 \times (-0.27 - 0.22^2) = 0.09 + 0.07^2$). However, this 'positive' effect results from late fledging individuals having yet to incur the lean tissue costs of moult. Perhaps this impending cost explains why we observed late fledging birds increasing lean tissue mass throughout the pre-migratory period, but did not see the same pattern for earlier fledging birds; late fledging birds may have been preemptively trying to mitigate the costs of moult associated with timing of nesting.

Moult requires substantial amounts of protein to replace the integument that supports feather growth [60,61]. However, most studies investigating the costs of moult in songbirds have taken place in the laboratory, where food is available *ad libitum* (e.g., [60,61]), making it difficult to assess how moult may affect lean tissue mass under natural conditions. Furthermore, when protein intake has been restricted, it is often at levels far below what would be encountered in the wild, and control groups are often still fed *ad libitum* (e.g., [77]). Our results suggest that moult progression may deplete pre-migratory lean tissue mass in wild birds. Similarly, Baggott [59] found that pectoralis muscle mass was negatively correlated with moult progression in free-living willow warblers *Phylloscopus trochilus*, and Bauchinger and Biebach [62] found that kidney and pancreas mass was negatively correlated with moult progression in garden warblers *Sylvia borin*. Our results and those of these other studies suggest the importance of assessing lean tissue costs of moult under natural conditions.

Our results suggest that the timing of nesting influences moult progression. Similar results have also been found for juvenile great tits *Parus major* and Savi's warblers *Locustella luscinioides* [66], [70]. This pathway represents another mechanism through which early life events may limit survival during migration, or even during the subsequent wintering period. For example, the rate of moult (not assessed here) may increase for late fledging individuals, resulting in lower quality plumage, which in turn may result in decreased flight performance and impaired thermoregulation [78–80]. Alternatively, delays in moult could constrain the timing of migratory departure [81]. To address these hypotheses in migratory species, improvements in tracking technologies are needed to follow individuals from birth and monitor subsequent survival rates at each stage of the annual cycle.

We found that pre-migratory total body water increased with date captured in 2008, but not in 2009 or 2010. Conversely, pre-migratory fat mass did not increase with date captured in 2008, but increased in both 2009 and 2010. These results suggest that individuals may be choosing to invest in either lean tissue mass or fat mass depending on current or anticipated conditions. For example, when food availability is unpredictable, songbirds tend to invest in fat mass [82,83]. Alternatively, annual differences in food availability or weather conditions may constrain what aspects of body condition are to be invested towards. In our study region, the average daily precipitation for the months of August and September were 4.0, 2.8, and 2.3 mm in 2008, 2009, and 2010 respectively (www.climate.weatheroffice.gc.ca). Juvenile songbirds are inefficient foragers, and rainstorms are expected to hamper their foraging abilities even further [84]. Therefore, the 'unpredictable foraging conditions' hypothesis predicts that individuals should invest in fat mass during 2008, however this is opposite to what we observe. Instead, increasing lean tissue mass in 2008 appears to be linked to precipitation through some other (unknown) causal mechanism.

Our results indicate that nestlings from larger broods had lower

4 , (**A**) ( ). Panels represent: (A) positive relationship between total body water and date captured for 2008, (B, C) shallow negative relationships between total body water and date captured for 2009 and 2010, respectively, (D) very shallow negative relationship between fat mass and date captured for 2008, (E, F) positive relationships between fat mass and date captured in 2009 and 2010, respectively. This figure suggests investment strategies in lean tissue mass or fat mass may vary depending on either current or anticipated environmental conditions. Date captured and year, were not included in models generating residuals for the plots. Trends illustrated with locally weighted regression lines.
(EPS)

1 (**1**) (**2**) (**= 3**) . A random effect was included for natal nest. Reference level for year is 2008. Parameter estimates based on standardized data.
(DOC)

2 **1** , (**1**) (**2**) (**= 188**) - . Random effects were included for natal nest, nested in mother, nested in year. ^{2} indicates curvilinear term. Parameter estimates based on un-standardized data.
(DOC)

3 **2009.** Both brood enlargements (n = 29 nestlings from n = 6 nests) and reductions (n = 26 nestlings from n = 9 nests) were carried out. One nestling from each treatment group was removed because tarsus length measurements were not obtained. Control nests were comprised of four nestlings. A random effect was included for natal nest. Parameter estimates based on un-standardized data.
(DOC)

4 , , , . ''Reduced'' and ''Control'' refer to experimental brood reductions (3 nestlings) and controls (4 nestlings), respectively. Numbers in parentheses represent original sample sizes prior to removing non-independent samples (see Methods: Model predictions and hypotheses).
(DOC)

5 - , (**1**) (**2**) (**= 33**). Random effects were included for individual nested within natal nest. Reference level for year is 2008. Parameter estimates based on standardized data.
(DOC)

6 - , - (**1**) (**2**) (**= 33**). Random effects were included for individual nested within natal nest. Reference level for year is 2008. Parameter estimates based on standardized data.
(DOC)

7 - , , (**1**) (**2**) (**= 33**). Random effects were included for individual nested within natal nest. ^{2} indicates curvilinear term. Reference level for year is 2008. Parameter estimates based on standardized data.
(DOC)

8 - (**1**) **2008** (**2**) **2009.** Nestlings were provided with a hypothesized immune system booster (lysozyme) or a hypothesized nutritional supplement (phosphate-buffered saline; PBS) every other day prior to day 8 (2008: n = 70 for PBS, n = 33 for lysozyme; 2009: n = 59 for PBS). Lysozyme was not used in 2009. Controls represent un-manipulated individuals. A random effect was included for natal nest. Experiments carried out by RA Mauck. Parameter estimates based on un-standardized data.
(DOC)

9 (**1**) (**2**) - (**B**) , , . Sample sizes in 2008: n = 27 from n = 12 nests for PBS, n = 14 from 4 nests for lysozyme. Sample sizes in 2009: n = 3 from n = 3 nests for PBS. A random effect was included for natal nest. Reference level for year is 2008. Parameter estimates based on standardized data.
(DOC)

10 - **B** (**1**) (**2**) (**= 5**). A random effect was included for natal nest. Reference level for year is 2009. Parameter estimates based on un-standardized data.
(DOC)

11 (, ;) , - , . 'X' denotes an interaction. The moult progression X year interaction for fat mass was no longer significant after the other interactions were removed from the model (year: 2009: $\beta = -0.54$, $t = -1.83$, DF = 47, P = 0.07; year: 2010: $\beta = -0.02$, $t = -0.11$, DF = 47, P = 0.91). Also, because the effect size and significance of interaction terms for date captured and year were similar in 2009 and 2010 for both total body water and fat mass, year was recoded as '2008' and '2009+2010' in order to reduce the number of parameters and paths included in the final path model. Random effects were included for individual nested within natal nest. Parameter estimates based on standardized data.
(DOC)

12 : (**1**) , (**2**) - , , , (**3**) - , , (**4**) - , . For number 1, a random effect was included for natal nest. For numbers 2 through 4, random effects were included for individual nested within natal nest. Reference level for year is 2008. ^{2} represents curvilinear term and 'X' denotes an interaction. Parameter estimates based on un-standardized data.
(DOC)

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Author Contributions

References

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