Physiological maturity at a critical life-history transition and flight ability at fledging

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Summary

1. Developmental maturity (e.g. body condition, body mass) at major life-history transitions is known to affect fitness across a wide range of taxa.

2. Fledging (leaving the nest), a major life-history transition in birds, is associated with high post-fledging mortality and is widely assumed to be related to poor initial flight ability of fledglings, which, in turn, might be related to developmental maturity at fledging.

3. We investigated individual variation in developmental maturity of both somatic and physiological traits at this critical life-history transition in different ecological contexts (year, first or second broods) to determine the importance of physiological traits related to oxygen-carrying capacity (haematocrit, haemoglobin) for individual variation in initial flight ability at fledging.

4. Haemoglobin concentration and haematocrit at fledging had much higher variance than somatic traits and were more variable across ecological contexts. Furthermore, fledgling haemoglobin concentration was the least developmentally mature of all traits (on average, only 78% of adult concentration).

5. Fledglings from second broods, which are known to have lower post-fledging survival, were less developmentally mature than fledglings from first broods for all traits (except tarsus), with haematocrit and haemoglobin concentration being the most developmentally immature traits (in first vs. second broods, haematocrit: 47·1% vs. 40·9%; haemoglobin: 13·3 vs. 11·6 g dL^{-1}).

6. Models predicting individual variation in two aspects of initial flight ability (total energy gain, take-off angle) were significantly improved when physiological traits (in particular haemoglobin) were incorporated into models based on somatic traits.

Key-words: developmental maturity, fledging, flight ability, haemoglobin, life-history transition, physiology

Introduction

Developmental maturity (e.g. body condition, mass) at major life-history transitions is known to affect individual fitness across a wide range of taxa (Clutton-Brock 1988; Green & Cockburn 2001; Altwegg & Reyer 2003), and conversely, selection on individuals can influence population-level changes via directional selection on overall size and developmental times (Kingsolver & Pfennig 2004). For example, pupal weight in insects correlates with higher fecundity later in life (Tammaru 1998), and experimental manipulation of larval environmental conditions explains variation in survival to reproductive maturity and adult mating success (De Block & Stoks 2005). In fish, hatching size and larval growth rate impact post-settlement survival (Shima & Findlay 2002; Vigliola & Meekan 2002), and larval size in marine invertebrates positively correlates

with post-metamorphic survival and fecundity (Dias & Marshall 2010). In passerine birds, the 'developmental milestone' when offspring progress from sedentary nestlings to active, free-flying fledgling is a critical life-history transition. Furthermore, the immediate period following fledging is marked by high mortality, with ≤50% of fledglings surviving in many species in the first 3–4 weeks after fledging (e.g. Anders *et al.* 1997; Adams, Skagen & Adams 2001; Rush & Stutchbury 2008). Predation is by far the most common cause of death in this post-fledging period, accounting for 55–80% of mortality (Kersten & Brenninkmeijer 1995; Anders *et al.* 1997; Adams, Skagen & Adams 2001), and in some studies, predation is the *only* cause of death recorded in the post-fledging period (Schmidt, Rush & Ostfeld 2008; Eng *et al.* 2011).

Poor flight performance of juveniles, and thus an inability to evade predators, is suggested as the reason for high mortality in the immediate post-fledging period (Sullivan 1989; Anders *et al.* 1997). However, few studies have

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directly measured flight performance at fledging, and little is known about the causes of individual variation in flight ability at this life-history transition or whether this relates to developmental maturity. Most studies have simply measured nestling mass or body size at fledging, without any assessment of flight metrics and correlate these somatic traits with survival. Although a significant positive relationship between late-stage nestling size and recruitment has been shown for many avian species, no relationship has been demonstrated in numerous other species (reviewed in Schwagmeyer & Mock 2008), even in species with high variability in fledgling mass (e.g. 200–400 g, Kersten & Brenninkmeijer 1995). Moreover, the majority of avian species fledge at or near adult body mass (Ricklefs 1968, 1979; Ricklefs & Peters 1979), but still have much higher juvenile mortality rates than adults. This suggests that other aspects of individual phenotype at fledging, such as physiological developmental maturity, might determine flight ability. Initial flight ability of fledglings has been linked to variation in wing area and wing loading (Verspoor *et al.* 2007; Chin *et al.* 2009), pectoral muscle mass (Veasey, Houston & Metcalfe 2000; Chin *et al.* 2009) and body mass:muscle ratio (Verspoor *et al.* 2007). However, few studies have considered physiological maturity at fledging (but see Vezina *et al.* 2009; Lill *et al.* 2013) especially as potential determinants of initial post-fledging flight ability. Here, we focus on developmental maturity of two physiological traits, haematocrit and haemoglobin, which can determine variation in aerobic capacity and VO_2max (Campbell 1995), oxygen off-loading and transfer from capillaries to mitochondria in muscle tissue (Wagner 1996), and matching of tissue oxygen delivery with local oxygen demand (Jensen 2009). Several studies have assumed that lower haematocrit negatively influences aerobic capacity and endurance flight performance (Saino *et al.* 1997; Hammond *et al.* 2000) although above-optimal increases in haematocrit can cause earlier exhaustion during physical activity and increased cardiopulmonary hypertension (Birchard 1997; Schuler *et al.* 2010). Although the few studies to date only link haematological metrics to endurance flight, we suggest that this might apply more generally to initial flight ability of fledglings given the rapid transition from sedentary nestlings to active, free-flying fledgling (see Discussion). Extensive individual variation in haematocrit has been documented in nestlings or chicks (Morrison, Ardia & Clotfelter 2009; Lill *et al.* 2013), and variation in haemoglobin concentration and haematocrit is correlated with pre-fledging survival (Nadolski *et al.* 2006; Kalinski *et al.* 2015) and post-fledging recruitment (Bowers *et al.* 2014), but no studies have related variation in these traits to individual variation in initial flight performance of fledglings. Finally, in free-living birds, heritability of haematocrit is low or zero (Potti 2007; Morrison, Ardia & Clotfelter 2009). This suggests that this is a phenotypically plastic trait with variation explained by the rearing environment (nest) during the developmentally sensitive growth phase (Potti 2007),

independent of fledging mass or condition (Morrison, Ardia & Clotfelter 2009). Thus, we predicted that physiological development of haematocrit and haemoglobin and fledging 'state' should depend on ecological context during rearing.

We measured haematocrit and haemoglobin concentration in chicks at fledging (day 21) in European starling *Sturnus vulgaris* chicks across two broods and years of differing productivity (ecological context), as well as measures of somatic development (wing length and wing area, body size and mass), and compared this to individual variation in the chick's initial flight performance. Specifically, we analysed individual variation in flight velocity, take-off angle and average mechanical energy produced in flight (measured following Verspoor *et al.* 2007) in relation to physiological versus somatic (mass/size) developmental maturity at fledging, with comparisons to adult values. We predicted (i) that physiological maturity and somatic maturity at fledging are less than adult values, and this would vary among years (i.e. for 'good' vs. 'bad' years), and potentially in a sex-specific manner, since males and females partition resources to physiological and somatic traits differently (Rowland *et al.* 2007; Verspoor *et al.* 2007; Schmidt, MacDougall-Shackleton & MacDougall-Shackleton 2012), (ii) that physiological and somatic trait values at fledging would be lower in second brood chicks vs. first brood chicks, given the former have higher immediate post-fledging mortality (Verboven & Visser 1998; Naef-Daenzer, Widmer & Nuber 2001), and (iii) that individual variation in initial flight performance (take-off angle, velocity, energy gain) would relate to not just somatic traits, but to the individual's maturity in physiological traits underlying oxygen-carrying capacity (haemoglobin, haematocrit).

Materials and methods

FIELD AND LABORATORY METHODS

We measured variation in chick physiological and somatic development at fledging in the European starling at Davistead Farm, Langley, British Columbia, Canada (49·17° N, 122·83° W), over 2 years of differing productivity (2013: 2·4 ± 1·8 chicks fledged vs. 2014: 3·7 ± 1·7 chicks fledged, long-term average 3·01 chicks, n = 15 years). This field site includes 150 nest boxes mounted on posts around pastures and on farm buildings and houses ~70 breeding pairs annually. Nest boxes were checked daily beginning in early April to determine the lay date of the first egg and clutch size. Nests were monitored until either failure or fledgling to quantify productivity. After the fledging of first broods, data collection was repeated for second broods. At fledging (day 21 day post-hatching), two chicks were removed from each nest box and placed in cloth bags until the flight performance trials (5–20 min). In 2014, we obtained detailed information on age of fledging: the majority of chicks (106/111, 95%) fledged on day 21 post-hatching with only 5/111 chicks fledging on day 20. Furthermore, only 8% of chicks hatched >24 h later than the first-hatched chicks within broods so there was little intrabrood variation in age of fledging.

The flight performance chamber consisted of a wooden frame 2·5 × 1 × 1 m covered with fine plastic netting (top, front and far end), plywood (bottom) and a white plastic back with a

10×10 cm grid. To ensure that take-off was independent of any handling effects at the time of release, birds were introduced into the chamber via a short (approx. 40 cm long) tube, 10 cm in diameter, and aligned at a 30° incline. Upon release, over 90 per cent of fledglings would launch into flight (after emerging from the tube) and would fly straight into the mesh at the end of the chamber (as though it was unseen). To prevent habituation to the process, fledglings were subjected to only two flight trials. Flight was videotaped with a digital video camera (JVC GZR10A Quad Proof Everio Full HD Camcorder; JVCKENWOOD USA Corporation, Long Beach, CA, USA; 30 frames per second) placed ~3 m from the front of the chamber. Following each flight, we immediately blood-sampled chicks and measured tarsus, mass and wing length (mean time between first flight and blood sampling <3 min). Time between flight trial and blood sampling (handling time) did not affect haemoglobin concentration ($t_{195} = 0.01$, $P = 0.930$) or mass ($t_{203} = -0.5$, $P = 0.596$), but was related to haematocrit ($t_{1,203} = -4.2$, $P < 0.001$). Time of day had no effect on mass ($t_{203} = 1.0$, $P = 0.336$), haematocrit ($t_{202} = 1.3$, $P = 0.186$), haemoglobin ($t_{196} = 1.6$, $P = 0.121$) or flight take-off angle ($t_{203} = 1.5$, $P = 0.124$), but did affect flight velocity ($t_{203} = 2.0$, $P = 0.048$) and total energy gain in flight ($t_{203} = 2.0$, $P = 0.047$). Where significant, minutes between flight and blood sampling (haematocrit) and time of day (total energy gain and flight velocity) were run as covariates in subsequent analyses. A digital photograph of the left wing, standardized in bent wing position against a scaled board, was taken to calculate wing area (using IMAGEJ, National Institutes of Health, Bethesda, MD, USA; Image Processing and Analysis in Java), which was used to calculate wing loading, that is the ratio of an individual's body weight to wing area. No wing area data were collected for second brood chicks in 2013 ($n = 31$) due to equipment malfunction, but wing length data were collected, which is positively correlated with wing area ($R_{177} = 0.49$). Blood samples were analysed for haematocrit and haemoglobin concentration as in the study by Williams *et al.* (2012). An aliquot of these samples were sent to Viaguard Accu-Metrics (Toronto, Ontario) for sexing by PCR. Adult values were determined by catching female parents of chicks during both 2013 and 2014 between 10 and 12 days after hatch via nest box traps, and blood sampling within 3 min of capture. These adult values were obtained just 9–11 days prior to chick fledging dates and thus represent adult values in the same environmental conditions (temperature, weather, prey availability, etc.) fledglings will encounter post-fledging. Female values were used for consistency, and due to low availability of male samples.

FLIGHT ANALYSIS

Flight trials were analysed on a computer using LOILOFIT Software (Dolby Laboratories, San Francisco, CA, USA). Vertical displacements (to the nearest 2·5 cm) and associated times (to the nearest quarter frame) were measured relative to the 10-cm grid at 0·5-m horizontal intervals, with the centre of the head used as reference. Flight time taken per interval was thus calculated as the number of frames $\times 1/30$ s frame^{-1}. Take-off velocity and angle were measured for the first horizontal 0·5 m 'take-off' component of the flight; however, the total energy gain metric included the energy produced during flight for the entire 2·5 m length of the chamber (see Discussion). We calculated average mechanical energy, E (J) for flight according to the equation from Williams & Swaddle (2003), $E = \frac{1}{2}(V_x^2 + V_z^2) + gz$ where V_x and V_z are the horizontal and vertical components of flight velocity, respectively; g is the acceleration due to gravity; and z is the height (Williams & Swaddle 2003). This measure was chosen since it describes both the height and velocity gain components of flight performance in a single variable (Williams & Swaddle 2003). In all cases, the best performance from the two trials was taken.

STATISTICAL ANALYSIS

All analyses were completed in R STUDIO version 0.98.1028 (R Studio Team 2015) using LSMEANS (Lenth 2016), MULTCOMP (Hothorn, Bretz & Westfall 2008), NLME (Pinheiro *et al.* 2016), AICcMODAVG (Mazerolle 2016) and STATS (R Studio Team 2015) packages. In the text portion of the results, means are presented as ±standard error of the mean. First, generalized linear models were used to compare female (due to availability) adult values with fledgling values for somatic and physiological traits, controlling for sex, brood and year as covariates unless stated otherwise. Variance was calculated on the per cent difference of the trait values from the mean trait value. Levene's test was used to compare variances between physiological traits (grouped) versus somatic traits (grouped). Next, linear mixed-effects models were used to evaluate the relationships between the somatic and physiological traits, and ecological context (year, brood) and sex. We used parent ID as random factor and controlled for handling time where significant (haematocrit). We repeated this analysis for flight metrics (controlling for time of day where significant).

Finally, we examined how somatic and physiological traits relate to flight performance in two steps. First, we determined the best model using somatic traits for each flight metric (take-off angle, flight velocity, total energy) controlling for time of day flown where significant (energy and velocity models). We included all possible combinations of somatic traits (tarsus, wing length, wing loading) in the candidate model set of linear mixed-effects models ($n = 8$ models, Table S1, Supporting Information). Models were assessed using Akaike's Information Criterion corrected for small sample sizes and ranked with ΔAIC values, with models ΔAIC < 2 considered strongly supported (Anderson & Burnham 2002). After we selected the best-supported somatic model for each flight metric, we subsequently asked whether the two physiological variables (together or individually) improved the best somatic model from earlier analysis ($n = 7$ models, Table 3).

Results

PHYSIOLOGICAL MATURATION AND SOMATIC MATURATION OF FLEDGLINGS COMPARED WITH ADULTS

Physiological traits (haematocrit, haemoglobin concentration) of fledglings were significantly below adult values, whereas not all somatic traits (mass, tarsus, wing loading and wing length) were different from adults. Fledgling body size measured as tarsus length was equal or relatively close to adult tarsus (93·3–100·5% of adult size; Table 1), though fledglings had 4·2% shorter tarsus lengths than female adults in 2014 ($F_{1,126} = 6·7$, $P = 0·011$) but not in 2013 ($F_{1,122} = 2·2$, $P = 0·145$). Though male fledglings were heavier than female fledglings (Table 2), mass was consistently less than adults ($F_{1,233} = 68·9$, $P < 0·001$), but averaged 86·0–90·1% of adult mass (Table 1). Fledgling wing length was the most developmentally immature of the somatic traits (Table 1) and significantly different from adults ($F_{1,214} = 220·4$, $P < 0·001$), but had relatively small individual variation in relative size (75–82% adult wing length). In contrast, the physiological traits had much higher variation in the level of developmental maturity among years, broods and sexes: fledgling haematocrit

averaged 73·2–93·4% of adult haematocrit and fledgling haemoglobin concentration averaged 66·5–84·4% of adult concentrations. Fledgling haemoglobin concentration ($F_{1,225} = 78·8$, $P < 0·001$) and haematocrit ($F_{1,233} = 51·8$, $P < 0·001$) were significantly below adult levels. The two physiological traits also had much higher variance (haematocrit: $\sigma^2 = 137$; haemoglobin: $\sigma^2 = 234$) than somatic traits (tarsus: $\sigma^2 = 39$; mass: $\sigma^2 = 71$; wing length: $\sigma^2 = 29$: contrast of grouped variance, $F_{1,1021} = 147·42$; $P < 0·001$).

YEAR, BROOD AND SEX AS SOURCES OF VARIATION IN PHYSIOLOGICAL MATURATION AND SOMATIC MATURATION

Year was the only a significant source of variation for some metrics, and almost exclusively in first brood chicks. For wing length, there was a year × brood interaction ($F_{1,156} = 4·5$; $P = 0·003$). Wing length was greater in 2014 compared with 2013 for first broods ($F_{1,148} = 6·7$; $P = 0·011$) but not for second broods ($F_{1,25} = 4·4$; $P = 0·057$). Consistent with the results for wing length, wing area was larger in 2014 ($60·5 \pm 0·8$ mm²) compared with 2013 ($49·9 \pm 0·7$ mm²) for first brood chicks ($F_{1,123} = 82·0$; $P < 0·001$). For tarsus, there was a main effect of year ($F_{1,193} = 14·6$; $P < 0·001$) and tarsus length was greater (+3·9%) in 2013 ($33·8 \pm 0·2$ mm) compared with 2014 ($32·5 \pm 0·2$ mm). However, both of these values were very similar to adult levels as described above (100·5% to 93·3% similarity to adults). Body mass was unrelated to year ($P > 0·05$). Among physiological traits, there was a year*brood interaction for fledgling haematocrit ($F_{1,209} = 14·7$; $P < 0·001$), controlling for minutes between flight and blood sampling for all analyses. Haematocrit was higher in first brood chicks in 2014 compared with 2013 ($F_{1,146} = 20·6$; $P < 0·001$), but with no difference in second brood chicks among years ($F_{1,34} = 1·0$; $P = 0·321$). Haemoglobin was not directly related to year ($P > 0·05$); however, there was a year × sex interaction ($F_{1,123} = 4·6$; $P = 0·035$). The only significant pairwise contrast was males with lower haemoglobin in 2014 compared with 2013 ($P < 0·010$).

Brood effects were significant for almost all traits, with first broods consistently more somatically and physiologically mature than second broods. Chicks from first broods were heavier ($71·6 \pm 0·5$ g, +3·9%) than second brood chicks ($68·7 \pm 0·9$ g; $F_{1,187} = 6·9$; $P = 0·009$). Wing length was greater in first broods compared with second broods in 2014 ($F_{1,99} = 41·0$; $P < 0·001$), but not in 2013 ($F_{1,64} = 2·4$; $P = 0·125$). Wing area was also larger in first brood chicks ($60·5 \pm 0·8$ mm²) compared with second brood chicks ($52·1 \pm 1·1$ mm²) in 2014 ($F_{1,91} = 18·8$; $P < 0·001$). Tarsus was the same for first and second broods ($P > 0·05$). Haematocrit was significantly higher in first brood ($47·1 \pm 0·4$%) compared with second brood ($40·9 \pm 0·7$%) in both years (2013: $F_{1,68} = 10·5$; $P = 0·002$; 2014: $F_{1,75} = 65·4$; $P < 0·001$). For haemoglobin concentration, there was a significant effect of brood number

Table 1. Percentage of fledgling value that corresponds to value of adult female during chick rearing, mean ± standard deviation, with %CV (coefficient of variation) given below in brackets. All year, brood and sex groups were significantly below adult value except for tarsus length

	2013				2014			
	Brood 1		Brood 2		Brood 1		Brood 2	
	Female (n = 32)	Male (n = 43)	Female (n = 13)	Male (n = 7)	Female (n = 37)	Male (n = 50)	Female (n = 12)	Male (n = 12)
Body mass	−10·7% ± 8·0	−11·2% ± 8·1	−14·4% ± 5·7	−10·8% ± 2·4	−12·1% ± 5·6	−10·0% ± 7·3	−19·5% ± 9·8	−11·6% ± 5·5
(%CV)	(9·0%)	(9·1%)	(6·7%)	(2·7%)	(6·4%)	(8·1%)	(12·2%)	(6·2%)
Tarsus	<0·0% ± 5·3	−0·9% ± 4·9	−0·8% ± 3·0	0·7% ± 3·8	−3·9% ± 5·2	−4·8% ± 7·6	−6·7% ± 6·6	0·6% ± 3·5
(%CV)	(5·3%)	(5·0%)	(3·0%)	(3·8%)	(5·4)	(8·0%)	(7·0%)	(3·5%)
Wing length	−19·8% ± 4·7	−20·8% ± 5·1	−22·2% ± 3·2	−19·6% ± 3·1	−17·8% ± 3·5	−18·6% ± 3·1	−25·2% ± 2·8	−21·4% ± 4·2
(%CV)	(5·8%)	(6·4%)	(4·1%)	(3·9%)	(4·3%)	(3·8%)	(3·8%)	(5·3%)
Haematocrit	−17·3% ± 9·1	−13·3% ± 7·9	−23·1% ± 8·9	−18·9 ± 4·9	−7·1% ± 7·4	−6·6% ± 7·4	−26·8% ± 13·3	−19·2% ± 4·8
(%CV)	(11·0%)	(9·1%)	(11·5%)	(6·0%)	(8·0%)	(7·9%)	(18·2%)	(5·9%)
Haemoglobin	−22·3% ± 14·5	−15·6% ± 10·9	−25·5% ± 13·1	−25·4 ± 9·5	−19·3% ± 9·2	−20·3% ± 8·8	−31·5% ± 17·1	−33·5% ± 9·2
(%CV)	(18·6%)	(12·9%)	(17·6%)	(12·8%)	(11·5%)	(11·0%)	(24·9%)	(13·8%)

Table 2. Summary of effects of year, brood and sex on somatic and physiological traits

Trait	Year	Brood	Sex
Body Mass	NS	Brood 1 > Brood 2	M > F
Tarsus	2013 > 2014	NS	NS
Wing Length	2014 > 2013 (Brood 1)	Brood 1 > Brood 2 (2014)	M > F (Brood 2)
Wing Area	2014 > 2013 (Brood 1)	Brood 1 > Brood 2 (2014)	NS
Haematocrit	2014 > 2013 (Brood 1)	Brood 1 > Brood 2	M > F
Haemoglobin	NS	Brood 1 > Brood 2	NS

($F_{1,186} = 28.8$; $P < 0.001$) and a marginally significant year × brood interaction ($F_{1,172} = 2.1$, $P = 0.050$), with higher concentration in first brood chicks (13.3 ± 0.1 g dL^{-1}) than second brood chicks (11.7 ± 0.3 g dL^{-1}).

Similar to year, sex effects were significant in few traits, with some interaction with brood number. Body mass was higher in males ($F_{1,159} = 7.3$; $P = 0.008$) and males on average were +2·4% heavier (71.8 ± 0.6 g males vs. 69.9 ± 0.6 g females). For wing length, there was a significant brood × sex interaction ($F_{1,160} = 9.5$; $P = 0.002$) and females had shorter wings than males in second broods ($F_{1,34} = 10.0$; $P = 0.003$) but not in 1st broods ($F_{1,132} = 0.75$; $P = 0.389$). Wing area was unrelated to sex ($P > 0.05$). Tarsus was not directly related to sex, but there was a significant brood × sex interaction ($F_{1,158} = 4.8$; $P = 0.029$) although no pairwise contrasts between brood 1 and 2 and male and female were significant (Bonferroni-corrected $P > 0.20$). Haematocrit was also higher on average in males (46.7 ± 0.4%) compared with females (44.6 ± 0.6%; $F_{1,167} = 9.8$; $P = 0.002$), but haemoglobin was not related to sex ($P > 0.05$).

INDIVIDUAL VARIATION IN PHYSIOLOGICAL MATURITY AND SOMATIC MATURITY AND INITIAL FLIGHT ABILITY

Take-off angle, velocity and energy gain were all unrelated to sex ($P > 0.05$ for full model). Take-off velocity was related to brood, with first broods flying faster (1.86 ± 0.03 m s^{-1}) than second broods (1.68 ± 0.04 m s^{-1}; $F_{1,190} = 12.2$, $P < 0.001$). Brood was marginally related to total energy gain during flight, with first broods producing more energy in flight (2.9 ± 0.2 J) than second broods (2.3 ± 0.3 J; $F_{1,195} = 2.7$, $P = 0.054$). Velocity was also related to year, with 2014 birds (1.68 ± 0.03 m s^{-1}) flying faster than 2013 birds (1.95 ± 0.03 m s^{-1}; $F_{1,190} = 12.2$, $P < 0.001$). However, birds in 2013 produced more energy in flight (3.6 ± 0.2 J) than in 2014 (2.1 ± 0.7 J; $F_{1,153} = 22.4$, $P < 0.001$). Take-off angle was not related to year or brood ($P > 0.05$ for full model).

Two of the three flight models were significantly improved by the addition of physiological traits to the basic somatic models. The best basic somatic model for total energy gain included wing length and wing loading,

but the addition of haemoglobin concentration to the model increased support by >100 times (w_i:w_i = <0·01:0·93) compared with the basic somatic model (Table 3). Similarly, the best-supported model for take-off angle included wing length, wing loading and haemoglobin, receiving 78% model support, with the model of wing length, wing loading, haemoglobin and haematocrit receiving 22% model support (Table 3). While the parameter estimate for concentration was low in the energy gain model (0.03 ± 0.07), the estimate was larger in the take-off angle model (1.06 ± 0.58). Unlike the other two flight metrics, the strongest velocity model included only the somatic traits wing loading and wing length, with models including physiological traits ranked second and third (Table 3).

Discussion

Fledging (leaving the nest), a major life-history transition in birds, is associated with high post-fledging mortality and is widely assumed to be related to poor initial flight ability of fledglings which, in turn, might be related to developmental maturity at fledging. We investigated individual variation in developmental maturity of both somatic and physiological traits at this critical life-history transition across different ecological contexts (years of below- and above-average productivity, first and second broods) to determine the importance of physiological traits related to oxygen-carrying capacity (haematocrit, haemoglobin) for individual variation in three measures of initial flight ability, a relevant trait for post-fledging survival. Chicks were developmentally immature at fledging for most somatic and physiological traits (though not body size measured as tarsus) with trait values significantly lower than adults. However, physiological traits were much more variable among individual chicks than somatic traits and were more variable across ecological contexts (year, brood), and haemoglobin concentration was the least developmentally mature of all traits (on average, only 78% of adult concentration). As predicted, second brood chicks, which have been shown to have lower post-fledging survival, were less developmentally mature than first brood chicks for all traits (except tarsus length), with haematocrit and haemoglobin concentration being the most developmentally immature traits in second brood chicks (on average in first vs. second broods, haematocrit: 47·1% vs. 40·9%; haemoglobin: 13·3 vs. 11·6 g dL^{-1}). Models predicting individual variation in two aspects of initial flight ability (total energy gain, take-off angle) were significantly improved when physiological traits (in particular haemoglobin) were incorporated into the models in addition to somatic traits (Table 3).

Numerous studies have shown that most birds fledge at, or very near adult body mass (Ricklefs 1968, 1979; Ricklefs & Peters 1979) and our data are consistent with this finding for body size, measured as mass and tarsus, both of which were relatively close to adult values in fledgling European starlings: average 88% adult mass, 97% adult

Table 3. Summary of AIC model outputs for models incorporating physiology, number of parameters: (*k*), AICc, ΔAICc, AICc weight (*w_i*) and log-likelihood

| Trait	Model	*k*	AIC$_c$	ΔAIC	*w_i*	Log-likelihood
Total energy gain	Somatic + Hb	8	669·08	0·00	0·93*	−326·09
	Somatic + Hb + Hct	9	674·16	5·08	0·07	−327·51
	Somatic Model	7	684·25	15·17	0·00	−334·79
	Somatic + Hct	8	689·88	20·80	0·00	−336·50
	Hb + Hct	7	816·49	147·41	0·00	−400·95
	Haemoglobin (Hb)	6	828·26	159·18	0·00	−407·91
	Haematocrit (Hct)	6	837·21	168·13	0·00	−412·39
Flight velocity	Somatic Model	7	106·46	0·00	0·74*	−45·94
	Haemoglobin (Hb)	7	109·24	2·79	0·18	−47·33
	Somatic + Hb	8	112·28	5·82	0·04	−47·76
	Haematocrit (Hct)	7	112·86	6·40	0·03	−49·14
	Somatic + Hct	8	116·02	9·57	0·01	−49·65
	Hb + Hct	8	117·77	11·31	0·00	−50·50
	Somatic + Hb + Hct	9	122·58	16·13	0·00	−51·81
Take-off angle	Somatic + Hb	6	1354·89	0·00	0·78*	−671·19
	Somatic + Hb + Hct	7	1357·43	2·55	0·22	−671·37
	Somatic Model	5	1404·06	49·17	0·00	−696·85
	Somatic + Hct	6	1405·32	50·43	0·00	−696·41
	Hb + Hct	5	1590·20	235·31	0·00	−787·81
	Haemoglobin (Hb)	4	1590·69	235·80	0·00	−791·24
	Haematocrit (Hct)	4	1648·17	293·28	0·00	−819·98

'Somatic' refers to the best model for each trait described in Table S1. 'Hb' refers to haemoglobin concentration, and 'Hct' refers to haematocrit.
*ΔAIC < 2.00.

tarsus length. However, fledglings showed marked developmental immaturity in wing length, with wings as low as 62% of adult length, though still more mature than physiological traits. This developmental immaturity is entirely consistent with the high mortality rate reported for fledglings (Krementz, Nichols & Hines 1989; Anders *et al.* 1997; Adams, Skagen & Adams 2001; Rush & Stutchbury 2008). Interestingly, while wing length was the most developmentally immature somatic trait (average 20% lower than adult lengths), this trait had the lowest individual variation of all traits. One possible explanation for this may be the optimization of wing loading (body mass/wing area). Wing loading has been shown to play an important role in escape flight in adult birds (Burns & Ydenberg 2002; McFarlane, Altringham & Askew 2016) and relates to flight performance in fledglings (Verspoor *et al.* 2007; Chin *et al.* 2009). Experimental manipulations using mass weights and feather clipping have already demonstrated that nestlings modify their somatic development in order to maintain targeted wing loading values at fledging (Wright, Markman & Denney 2006). In fact, European starling chicks lose mass prior to fledging (so lower body mass at fledging does not reflect lack of development *per se* and peak chick mass is closer to adult mass) and this decrease in mass, combined with smaller wing size, would actually maintain wing loading closer to the adult ratio. In addition, given that fledgling starlings undergo a complete pre-basic moult replacing all their flight feathers just 5–6 weeks after fledging, it may be disadvantageous to invest in feather growth over other development (Feare 1984).

Simmons & Lill (2006) showed that pre-fledging mass recession was paralleled by a decrease in oxygen-carrying capacity of blood, measured as haemoglobin concentration, in the welcome swallow *Hirundo neoxena* which they suggested might reflect a drop in metabolic rate associated with lower growth rates of older chicks. However, there is no pre-fledging decrease in haemoglobin or haematocrit in European starlings (A. Cornell, in prep.), suggesting that there might be species-specific variation in the timing and trajectory of physiological developmental maturity approaching fledging.

We predicted a strong negative relationship between brood number and maturity at fledging, given that many other studies have documented lower survival and recruitment of second brood offspring (Verboven & Visser 1998; Naef-Daenzer, Widmer & Nuber 2001). This relationship was true for all somatic traits except tarsus length, which was already similar to adult levels, consistent with other studies comparing chick quality declining with date (Krementz, Nichols & Hines 1989; Naef-Daenzer, Widmer & Nuber 2001). We also found some sexual dimorphism in fledglings, similar to prior studies (Verspoor *et al.* 2007), which may suggest differential investment strategies on the part of provisioning parent birds (Stamps 1990; Magrath *et al.* 2004; Mainwaring, Lucy & Hartley 2011), or sex-specific developmental trajectories of the chicks themselves (Riedstra, Dijkstra & Daan 1998; Vedder *et al.* 2005). (Fig. 1). We found annual variation in developmental maturity for wing length, wing area, tarsus and haematocrit, but these effects were influenced by interactions with
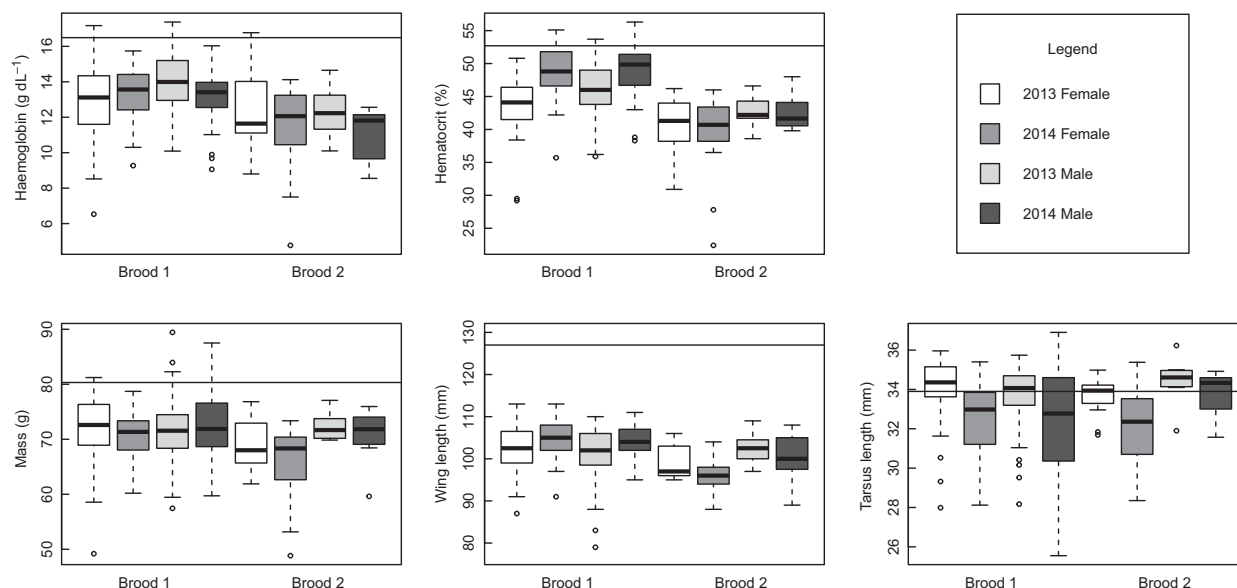
Fig. 1. Physiological and somatic trait values in fledglings from 2013 and 2014 of both broods and sexes. Horizontal line represents average value for adult female during chick rearing.

brood. In general, these effects were in the direction we would have predicted: chicks were more developmentally immature at fledging in 2013, a 'poor' year based on overall breeding productivity. Year effects may have been weaker than brood effects if parents adjust their investment to optimize chick quality in low quality years; that is fewer chicks raised in 2013 (mean 2·4 chicks fledged vs. 3·7 in 2014), but chicks still fledged at similar mass.

In contrast to the somatic traits, the values of physiological traits (haemoglobin, haematocrit) were both substantially lower than adults (only 78% of adult values for haemoglobin) *and* had significantly higher individual variation. However, we found that developmental maturity in physiology is context dependent (Table 2). First brood fledglings had higher haematocrit and haemoglobin concentration, and fledglings from the high productivity year also had higher haematocrit, although not haemoglobin. In contrast to our results, other studies have found significant year effects on chick haemoglobin. However, these studies measured haemoglobin concentration earlier in development: 6–7 days before fledging (Bańbura *et al.* 2007), 5 days prior to fledging (Gladalski *et al.* 2016) and 4–7 days prior to fledging (Kalinski *et al.* 2015), whereas our study measured chicks on the day of fledging when chicks from different quality years may have converged. Other studies have also found ecological context to influence haematocrit and haemoglobin concentration in the form of habitat quality (Bańbura *et al.* 2007; Kalinski *et al.* 2015), parasite load (Słomczyński *et al.* 2006), elevation (Fair, Whitaker & Pearson 2007) and season (Fair, Whitaker & Pearson 2007). Furthermore, there is accumulating evidence for fitness effects of haemoglobin concentration in adults. For example, within individuals, migrating birds have been shown to adjust their

haemoglobin levels at stopover sites in preparation for long distance flights (Minias *et al.* 2014) and among species longer distance migrants have higher concentrations of haemoglobin than short distance migrants (Minias *et al.* 2013). During the breeding season, haemoglobin concentration has been positively correlated with number of chicks fledged in female European starlings, and experimental decrease in haemoglobin decreased chick mass at fledging (Fronstin, Christians & Williams 2015). Given that fledglings are transitioning to a high activity, volant lifestyle, the development of physiological traits underlying aerobic capacity may be critical to surviving this transition.

The somatic traits we measured produced models of flight performance similar to Verspoor *et al.* (2007), and Chin *et al.* (2009), but when we incorporate physiology into the best somatic models for take-off angle and total energy gain in flight, physiological traits significantly improved the models (energy gain: $w_i:w_i = <0\cdot01:0\cdot93$; take-off angle: $w_i:w_i = <0\cdot01:0\cdot78$, Table 3). The best models for take-off angle and energy gain included haemoglobin concentration, which was our most developmentally immature and most individually variable trait. Haemoglobin concentration has been proposed as a robust indicator of physiological condition in birds (reviewed in Minias 2015), and unlike the somatic traits, haemoglobin concentration can indicate high oxygen-carrying capacity of blood necessary for high levels of aerobic activity (Campbell 1995). Thus, our study identifies a potential physiological mechanism linking developmental maturity at fledging, post-fledging flight ability and survival that has been documented in other studies (Nadolski *et al.* 2006; Bowers *et al.* 2014; Kalinski *et al.* 2015). We acknowledge that we only measured the first two flights each fledgling

made and that our measurement of flight ability is limited by the dimensions of the flight chamber (2·5 m long). However, we used three measures of initial flight ability and one of these (total energy gain) is somewhat more 'sustained' (at least over 2·5 m) than the immediate vertical take-off escape component. In fact, most previous studies (e.g. Veasey, Metcalfe & Houston 1998; Kullberg, Houston & Metcalfe 2002; Williams & Swaddle 2003) measured true 'take-off ability' in adult birds as an acute, upward take-off angle in a vertically orientated flight chamber. Verspoor *et al.* (2007) showed that fledgling starlings were incapable of sustained vertical take-off, hence the use of a horizontal flight chamber in theirs, and our, study. In addition, we did not use a 'startle stimulus' (e.g. a loud bang) to initiate flight, as is the case in most other studies of adult escape flights (see Verspoor *et al.* 2007). Clearly, initial take-off ability is important for evading predators, but it is not the only key component of flight. For example, the ability to sustain power after initial take-off (measured as total energy gain over 2·5 m in our study) could be important, and we think it is plausible that this might correlate with, or predict, initial post-fledging flight ability even over longer duration flights in the days after fledging (and which might affect non-predator mediated components of fitness, e.g. foraging ability). We suggest that sustained flights, that is endurance flight, may depend even more on determinants of aerobic capacity such as haemoglobin and haematocrit in the immediate post-fledging period. Future studies should therefore assess different measures of post-fledging flight ability (though this will be complicated as the time required to train birds for endurance flights, e.g. in a wind tunnel, might mean that physiology at the time of testing could be very different from fledging physiology).

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.c2n66 (Cornell, Gibson & Williams 2016).

References

Adams, A.A., Skagen, S.K. & Adams, R.D. (2001) Movements and survival of lark bunting fledglings. *Condor*, **103**, 643–647.

Altwegg, R. & Reyer, H.U. (2003) Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, **57**, 872–882.

Anders, A.D., Dearborn, D.C., Faaborg, J. & Thompson, F.R.I. (1997) Juvenile survival in a population of neotropical migrant birds. *Conservation Biology*, **11**, 698–707.

Anderson, D.R. & Burnham, K.P. (2002) Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management*, **66**, 912–918.

Bańbura, J., Bańbura, M., Kaliński, A., Skwarska, J., Słomczyński, R., Wawrzyniak, J. *et al.* (2007) Habitat and year-to-year variation in haemoglobin concentration in nestling blue tits *Cyanistes caeruleus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **148**, 572–577.

Birchard, G.F. (1997) Optimal hematocrit: theory, regulation and implications. *American Zoologist*, **37**, 65–72.

Bowers, K.E., Hodges, C.J., Forsman, A.M., Vogel, L.A., Masters, B.S., Johnson, B.G. *et al.* (2014) Neonatal body condition, immune responsiveness, and hematocrit predict longevity in a wild bird population. *Ecology*, **95**, 3027–3034.

Burns, J. & Ydenberg, R. (2002) The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behavioral Ecology and Sociobiology*, **52**, 128–136.

Campbell, T.W. (1995) *Avian Hematology and Cytology*. Iowa State University Press, Iowa City, IA, USA.

Chin, E.H., Love, O.P., Verspoor, J.J., Williams, T.D., Rowley, K. & Burness, G. (2009) Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proceedings of Royal Society of London B: Biological Sciences*, **276**, 499–505.

Clutton-Brock, T.H. (1988) *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*. University of Chicago Press, Chicago, IL, USA.

Cornell, A., Gibson, K.F. & Williams, T.D. (2016) Data from: Physiological maturity at a critical life-history transition and flight ability at fledging. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.c2n66.

De Block, M. & Stoks, R. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology*, **86**, 185–197.

Dias, G.M. & Marshall, D.J. (2010) Does the relationship between offspring size and performance change across the life-history? *Oikos*, **119**, 154–162.

Eng, M.L., Stutchbury, B.J., Burke, D.M. & Elliot, K.A. (2011) Influence of forest management on pre- and post-fledging productivity of a Neotropical migratory songbird in a highly fragmented landscape. *Canadian Journal of Forest Research*, **41**, 2009–2019.

Fair, J., Whitaker, S. & Pearson, B. (2007) Sources of variation in haematocrit in birds. *Ibis*, **149**, 535–552.

Feare, C. (1984) *The Starling*. Oxford University Press, Oxford, UK.

Fronstin, R.B., Christians, J.K. & Williams, T.D. (2015) Experimental reduction of haematocrit affects reproductive performance in European starlings. *Functional Ecology*, **30**, 398–409.

Gladalski, M., Bańbura, M., Kalinksi, A., Markowski, M., Skwarska, J., Wawrzynaik, J. *et al.* (2016) Spatial Variation in haemoglobin concentration of nestling blue tits (*Cyanistes caeruleus*): a long-term perspective. *Journal of Ornithology*, **157**, 591–598.

Green, D.J. & Cockburn, A. (2001) Post-fledging care, philopatry and recruitment in brown thornbills. *Journal of Animal Ecology*, **70**, 505–514.

Hammond, K.A., Chappell, M.A., Cardullo, R.A., Lin, R.S. & Johnsen, T.S. (2000) The mechanistic basis of aerobic performance variation in red junglefowl. *Journal of Experimental Biology*, **203**, 2053–2064.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Jensen, F.B. (2009) The dual roles of red blood cells in tissue oxygen delivery: oxygen carriers and regulators of local blood flow. *Journal of Experimental Biology*, **212**, 3387–3393.

Kalinski, A., Bańbura, M., Gladalski, M., Markowski, M., Wkwarska, J., Wawrzyniak, J. *et al.* (2015) Long-term variation in hemoglobin concentration in nestling great tits *Parus major*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **185**, 9–15.

Kersten, M. & Brenninkmeijer, A. (1995) Growth, fledging success and post-fledgling survival of juvenile oystercatchers *Haematopus ostralegus*. *Ibis*, **137**, 396–404.

Kingsolver, J.G. & Pfennig, D.W. (2004) Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution*, **58**, 1608–1612.

Krementz, D.G., Nichols, J.D. & Hines, J.E. (1989) Postfledging survival of European starlings. *Ecology*, **70**, 646–655.

Kullberg, C., Houston, D.C. & Metcalfe, N.B. (2002) Impaired flight ability – a cost of reproduction in female blue tits. *Behavioral Ecology*, **13**, 575–579.

Lenth, R.V. (2016) Least-squares means: the R package lsmeans. *Journal of Statistical Software*, **69**, 1–33.

Lill, A., Rajchl, K., Yachou-Wos, L. & Johnstone, C.P. (2013) Are hematocrit and hemoglobin concentration reliable body condition indicators in nestlings: the welcome swallow as a cast study. *Avian Biology Research*, **6**, 57–66.

Magrath, M.J., van Lieshout, E., Visser, G.H. & Komdeur, J. (2004) Nutritional bias as a new mode of adjusting sex allocation. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**(Suppl 5), S347–S349.

Mainwaring, M.C., Lucy, D. & Hartley, I.R. (2011) Parentally biased favouritism in relation to offspring sex in zebra finches. *Behavioral Ecology and Sociobiology*, **65**, 2261–2268.

Mazerolle, M.J. (2016) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-4. http://CRAN.R-project.org/package=AICcmodavg.

McFarlane, L., Altringham, J.D. & Askew, G.N. (2016) Intra-specific variation in wing morphology and its impact on take-off performance in blue tits (*Cyanistes caeruleus*) during escape flights. *Journal of Experimental Biology*, **219**, 1369–1377.

Minias, P. (2015) The use of haemoglobin concentrations to assess physiological condition in birds: a review. *Conservation Physiology*, **3**, cov007.

Minias, P., Kaczmarek, K., Włodarczyk, R. & Janiszewski, T. (2013) Hemoglobin concentrations in waders vary with their strategies of migration: a comparative analysis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **65**, 7–12.

Minias, P., Włodarczyk, R., Piasecka, A., Kaczmarek, K. & Janiszewski, T. (2014) Ecological, physiological, and morphological correlates of blood hemoglobin concentration in a migratory shorebird. *Physiological and Biochemical Zoology*, **87**, 771–781.

Morrison, E.S., Ardia, D.R. & Clotfelter, E.D. (2009) Cross-fostering reveals sources of variation in innate immunity and hematocrit in nestling tree swallows *Tachycineta biocolor*. *Journal of Avian Biology*, **40**, 573–578.

Nadolski, J., Skwarska, J., Kaliński, A., Bańbura, M., Śniegula, R. & Bańbura, J. (2006) Blood parameters as consistent predictors of nestling performance in great tits (*Parus major*) in the wild. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **143**, 50–54.

Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology*, **70**, 730–738.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2016) nlme: Linear and nonlinear mixed effects models. R package version 3.1-128.

Potti, J. (2007) Variation in the hematocrit of a passerine bird across life stages is mainly of environmental origin. *Journal of Avian Biology*, **38**, 726–730.

R Studio Team (2015) *RStudio: Integrated Development for R*. RStudio, Inc., Boston, MA, USA. http://www.rstudio.com/.

Ricklefs, R.E. (1968) Patterns of growth in birds. *Ibis*, **110**, 419–451.

Ricklefs, R.E. (1979) Patterns of growth in birds. V. A comparative study of development in the starling, common tern, and Japanese quail. *The Auk*, **96**, 10–30.

Ricklefs, R.E. & Peters, S. (1979) Intraspecific variation in the growth rate of nestling European starlings. *Bird-Banding*, **50**, 338–348.

Riedstra, B., Dijkstra, C. & Daan, S. (1998) Daily energy expenditure of male and female marsh harrier nestlings. *The Auk*, **115**, 635–641.

Rowland, E., Love, O.P., Verspoor, J.J., Sheldon, L. & Williams, T.D. (2007) Manipulating rearing conditions reveals developmental sensitivity in the smaller sex of a passerine bird, the European starling *Sturnus vulgaris*. *Journal of Avian Biology*, **38**, 612–618.

Rush, S.A. & Stutchbury, B.J. (2008) Survival of fledgling Hooded Warblers (*Wilsonia citrina*) in small and large forest fragments. *The Auk*, **125**, 183–191.

Saino, N., Cuervo, J.J., Krivacek, M., de Lope, F. & Moller, A.P. (1997) Experimental manipulation of tail ornament size affects the hematocrit of male barn swallows (*Hirundo rustica*). *Oecologia*, **110**, 186–190.

Schmidt, K.L., MacDougall-Shackleton, E.A. & MacDougall-Shackleton, S.A. (2012) Developmental stress has sex-specific effects on nestling growth and adult metabolic rates but no effect on adult body size or body composition in song sparrows. *Journal of Experimental Biology*, **215**, 3207–3217.

Schmidt, K.A., Rush, S.A. & Ostfeld, R.S. (2008) Wood thrush nest success and post-fledging survival across temporal pulse of small mammal abundance in an oak forest. *Journal of Animal Ecology*, **77**, 830–837.

Schuler, B., Arras, M., Keller, S., Rettich, A., Lundby, C., Vogel, J. *et al.* (2010) Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin-treated mice. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 419–423.

Schwagmeyer, P.L. & Mock, D.W. (2008) Parental provisioning and offspring fitness: size matters. *Animal Behavior*, **75**, 291–298.

Shima, J.S. & Findlay, A.M. (2002) Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. *Marine Ecology Progress Series*, **235**, 303–309.

Simmons, P. & Lill, A. (2006) Development of parameters influencing blood oxygen carrying capacity in the welcome swallow and fairy martin. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **143**, 459–468.

Słomczyński, R., Kaliński, A., Wawrzyniak, J., Bańbura, M., Skwarska, J., Zieliński, P. *et al.* (2006) Effects of experimental reduction in nest micro-parasite and macro-parasite loads on nestling hemoglobin level in blue tits *Parus caeruleus*. *Acta Oecologica*, **30**, 223–227.

Stamps, J.A. (1990) When should avian parents differentially provision sons and daughters? *The American Naturalist*, **135**, 671–685.

Sullivan, K.A. (1989) Predation and starvation: age-specific mortality in juvenile juncos (*Junco phaenotus*). *Journal of Animal Ecology*, **58**, 275–286.

Tammaru, T. (1998) Determination of adult size in a folivorous moth: constraints at instar level? *Ecological Entomology*, **23**, 80–89.

Veasey, J.S., Metcalfe, N.B. & Houston, D.C. (1998) A reassessment of the effect of body mass upon flight speed and predation risk in birds. *Animal Behaviour*, **56**, 883–889.

Veasey, J.S., Houston, D.C. & Metcalfe, N.B. (2000) Flight muscle atrophy and predation risk in breeding birds. *Functional Ecology*, **14**, 115–121.

Vedder, O., Dekker, A.L., Visser, G.H. & Dijkstra, C. (2005) Sex-specific energy requirements in nestlings of an extremely sexually size dimorphic bird, the European sparrowhawk (*Accipiter nisus*). *Behavioral Ecology and Sociobiology*, **58**, 429–436.

Verboven, N. & Visser, M.E. (1998) Seasonal variation in local recruitment of great tits: the importance of being early. *Oikos*, **81**, 511–524.

Verspoor, J.J., Love, O.P., Rowland, E., Chin, E.H. & Williams, T.D. (2007) Sex-specific development of avian flight performance under experimentally altered rearing conditions. *Behavioral Ecology*, **18**, 967–974.

Vezina, F., Love, O.P., Lessard, M. & Williams, T.D. (2009) Shifts in metabolic demands in growing altricial nestlings illustrate context-specific relationships between basal metabolic rate and body composition. *Physiological and Biochemical Zoology*, **82**, 248–257.

Vigliola, L. & Meekan, M.G. (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia*, **131**, 89–93.

Wagner, P.D. (1996) Determinants of maximal oxygen transport and utilization. *Annual Review of Physiology*, **58**, 21–50.

Williams, E.V. & Swaddle, J.P. (2003) Moult, flight performance and wing-beat kinematics during take-off in European starlings. *Journal of Avian Biology*, **34**, 371–378.

Williams, T.D., Fronstin, R.B., Otomo, A. & Wagner, E. (2012) Validation of the use of phenylhydrazine hydrochloride (PHZ) for experimental manipulation of haematocrit and plasma haemoglobin in birds. *Ibis*, **154**, 21–29.

Wright, J., Markman, S. & Denney, S.M. (2006) Facultative adjustment of pre-fledging mass loss by nestling swifts preparing for flight. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 1895–1900.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Summary of AIC model outputs on somatic traits, includes number of parameters: (k), AICc, ΔAICc, AICc weight (w_i), and log-likelihood.