Development, pre-fledging physiological state, and post-fledging performance in the European starling, *Sturnus vulgaris*

by

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Abstract

Body size (mass, wing length) is typically used as a metric of developmental state and physiological quality in juvenile birds. However, our understanding of non-linear patterns of development (e.g., mass-overshoot recession) in relation to actual physiological state is poor, especially approaching fledging. We explored covariation between pre-fledging morphological development (body mass, tarsus, and wing length) and physiological state at fledging (aerobic capacity, energy state, oxidative status) in the European starling (*Sturnus vulgaris*). Developmental trajectories consistent with pre-fledging mass recession and compensatory wing growth were negatively associated with energy state and aerobic capacity, respectively. In contrast, no measure of body size covaried with any single physiological trait, although fledgling mass was positively correlated with principal component scores that comprised aerobic capacity and energy state. Next, we used a weight-treatment experiment to test how putative facultative adjustments in body mass prior to fledging affect growth and physiological state, using radiotelemetry data to subsequently analyze post-fledging performance. Only body mass and oxidative status differed between treatments, suggesting mass-independent development of some physiological traits but oxidative costs for maintaining developmental trajectories despite experimental perturbations. Both morphology (mass recession, wing growth and length) and physiological state (haemoglobin, reactive oxygen metabolites) were associated with metrics of post-fledging performance in control birds, although no trait predicted performance in the treatment group. To further test the apparent insensitivity of physiological development to environmental perturbations, we conducted a brood size manipulation and cross-fostering experiment. Results showed structural and physiological development that was robust to environmental variation in two growth phases. Again, this apparent canalization was associated with oxidative costs in low-quality environments, which carried over between growth phases even when conditions improved. Finally, we explored the role of behaviour in pre-fledging development by quantifying two pre-fledging ‘exercises’: wing extensions and wing flapping. We tested for covariation between these exercises and development, showing independent relationships with chick age, morphology, and provisioning rate, perhaps suggestive of separate adaptive functions. Collectively, this work suggests that some measures of physiological state independently vary with environmental context and performance, and this variation might affect juvenile performance independent of absolute body size.

Keywords: development; physiological state; post-fledging performance; canalization; exercise

Dedication

To my wife Alice, without whom this thesis would have never been written. Though she will never read it, I’m sure we’ll chat about it once or twice. As long as I make her a cup of tea first.

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Preface

Physiological mechanisms that underpin variation in life-history traits and trade-offs remain critically understudied. Assessments of individual quality often solely include indices of body size (i.e., morphological traits), typically assumed to reflect physiological state, although empirical evidence supporting this in juveniles is lacking. While adult size may reflect physiological quality, few studies have considered how non-linear, potentially flexible, patterns of development (e.g., pre-fledging mass recession) may affect putative links between morphological and physiological traits in juveniles. In contrast, recent studies have shown a surprising *in*flexibility, or insensitivity, of some morphological developmental trajectories (e.g., wing growth) in response to environmental variation. Yet, how physiological state relates to these ‘canalized’ morphological traits remains uncertain, while the potential canalization of physiological traits has rarely been tested. Exploring how physiological development relates to body size is therefore instrumental for our understanding and definitions of ‘high’ versus ‘low’ quality juveniles, especially considering the high rates of post-fledging mortality in many avian taxa. Current evidence for what determines individual variation in survival through the post-fledging period is equivocal, but few studies have tested the importance of physiological state for immediate fledgling performance.

This thesis therefore aimed to make a more holistic determination of what developmental factors influence individual quality in juvenile birds, primarily by considering a suite of physiological traits (aerobic capacity, energy state, oxidative status), but also by examining pre-fledging nestling behaviour and post-fledging performance. Specifically, this thesis consists of four data-chapters where we investigated covariation between morphological and physiological traits in European starlings (*Sturnus vulgaris*) approaching fledging (Chapter 1), tested causal relationships between pre-fledging mass recession and physiological state, in addition to examining correlations with post-fledging performance (Chapter 2), tested the sensitivity of morphological and physiological traits to environmental variation (Chapter 3), and explored the importance of nestling ‘exercise’ for pre-fledging morphological development (Chapter 4).

We used the European starling (*Sturnus vulgaris*) as our study species, which notably demonstrates a mass-overshoot recession growth profile. Here, nestlings achieve adult mass relatively early in development, only to lose a significant portion of that mass prior to fledging. This developmental trajectory is not unique to European starlings, however, as it has been documented in other passerines, as well as seabirds and raptors. Yet, species-specific growth profiles are rarely considered when assessing individual quality in juvenile birds, despite developmental patterns like mass-overshoot recession having strong implications for the use of body mass as a metric of condition. Therefore, by using European starlings, this thesis could explore potential causes and consequences of individual variation in physiological development under the constant theme of mass recession, questioning traditional biological views relating to growth, size, and maturity.

In Chapter 1 of this thesis, we examined how pre-fledging developmental patterns, like mass recession, relate to both size and physiological state at fledging in a natural, unmanipulated setting. By measuring (body mass, tarsus, wing length) chicks in the approach to fledging, and blood sampling them at fledging, we were able to test the assumption that juvenile size reflects physiological state, while exploring the growth trajectories that may influence correlations between morphological and physiological traits. This was done both by testing for individual, potentially mechanistic, relationships between morphological and physiological traits, and by creating a principal component representing overall ‘physiological quality’ that could be correlated to body size.

Chapter 2 involved an experimental manipulation of pre-fledging mass recession to test for causative relationships between juvenile body mass and physiological state. At asymptotic mass, we fitted nestlings with 4.0 g (approx. ~5% body mass) weighted backpacks and predicted a facultative adjustment of mass recession in response, as shown previously in swifts. Again, we measured weight-treated and control chicks in the approach to fledging and assessed physiological state at fledging. Specifically, we focused on the potential physiological mechanisms of mass recession, its association with physiological maturation, and if it has any associated physiological costs. We also fitted previously weighted and control chicks with radiotransmitters at fledging, exploring the effects of physiological state, as well as weight-treatment, on post-fledging performance (activity, activity-slope, day of dispersal). Since the focus of Chapter 2 was on pre-fledging mass trajectories, we sought to manipulate development further in Chapter 3 to test more generally (both in terms of developmental stage and the traits manipulated) how variation in morphological traits, due to differences in environmental quality, might reflect physiological state.

Chapter 3 included both a brood-size manipulation and cross-fostering experiment. Chicks were placed in enlarged or reduced broods at hatch and were subsequently swapped between broods in the approach to fledging. Measurements and blood samples were taken at asymptotic mass on day 15, immediately prior to cross-fostering, and again on day 20, five days after cross-fostering. By assessing morphological and physiological state on day 15, we tested for the initial effects of high versus low quality natal environments on 'early' nestling development, while repeating the same measures on day 20 allowed us to test how a change in environmental quality might affect some of the 'late' pre-fledging developmental patterns observed in Chapter 1 and 2 (e.g., mass recession, compensatory wing growth). While the experiment was initially designed to manipulate development to test for causal associations between morphological and physiological traits, Chapter 3 also focused on canalization, whereby certain traits demonstrate a developmental robustness to environmental perturbations.

Chapter 4 explored the importance of nestling 'exercise' for pre-fledging developmental patterns identified in prior chapters. While anecdotal accounts of 'flight preparation' behaviours are common in seabirds (e.g., wing flapping), seldom are they quantified, and the prevalence of such 'exercise' in passerine birds is unknown. By placing cameras in nestboxes of European starlings, we were able to quantify exercise in the nest immediately prior to nest departure and test its importance for pre-fledging morphological development. Most notably, it was previously suggested that increased activity levels prior to fledging could be a mechanism for pre-fledging mass recession, which Chapter 4 explores.

Collectively, this thesis culminates three years of morphological, physiological, and behavioural data pertaining to pre-fledging development and post-fledging performance in the European starling. This research encompasses a comprehensive analysis of the individual and general correlations between morphological and physiological traits (Chapter 1), the first test of physiological state in relation to an experimental manipulation of pre-fledging mass recession (Chapter 2), the first study of post-fledging activity in relation to fledgling physiology (Chapter 2), the first use of a combined brood-size manipulation and cross-fostering experiment to explore physiological canalization (Chapter 3), and the first quantification of pre-fledging ‘exercise’ in a passerine bird (Chapter 4).

Chapter 1.

Flexible growth and body mass predict physiological condition at fledging in the synchronously breeding European starling, *Sturnus vulgaris*

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1.1. Chapter abstract

Recent studies have reported beneficial carryover effects of juvenile development that predict interspecific survival differences at independence. Yet, traits relating to body size (i.e., morphological traits) have proven to be unreliable predictors of juvenile survival within species. Exploring individual variation of growth trajectories and how they covary with physiology could reveal species-specific developmental modes which have implications for our assessments of juvenile quality. Here, we investigated morphological development of European starlings (*Sturnus vulgaris*) approaching fledging in relation to three components of physiological condition at independence: aerobic capacity, energy state, and oxidative status. We found evidence of flexible mass and wing growth which independently covaried with fledgling energy state and aerobic capacity, respectively. By comparison, tarsus and wing length at fledging were unrelated to any physiological trait, whilst mass was positively associated with principal component scores that comprised aerobic capacity and energy state. Thus, flexible growth trajectories were consistent with 'developmental plasticity': adaptive pre-fledging mass recession and compensatory wing growth, which seemingly came at a physiological cost, whilst fledgling body mass positively reflected overall physiological condition. This highlights how patterns of growth and absolute size may differently reflect fledgling physiology, potentially leading to variable relationships between morphological traits and juvenile fitness.

1.2. Introduction

Ontogenetic growth and fitness are intimately related due to strong effects of body size on fecundity and longevity (Williams, 1957). In birds and mammals, size at maturity is a key determinant of future survival (reviewed in Ronget *et al*., 2018) and predicts that there should be strong selection to prioritize growth or developmental maturity of any trait that contributes to fitness (Waddington, 1942; Cheng and Martin, 2012; Boonekamp *et al*., 2017, 2018). In birds, differences in developmental maturity at fledging (nest departure) have been used to explain variation in post-fledging mortality across taxa (Maness and Anderson, 2013; Martin *et* al., 2018; Jones and Ward, 2020). However, our understanding of developmental maturity in birds is often limited to measures of absolute body size, typically mass and/or structural size (e.g., wing length), restricting our understanding of individual quality (*sensu* Wilson and Nussey, 2010) to predominantly morphological traits (i.e., relating to chick size). Morphological traits have proven useful in understanding variation in rates of juvenile mortality between species (Martin *et al*., 2018; Jones and Ward, 2020), but have produced mixed results within species (Magrath, 1991; Schwagmeyer and Mock, 2008; Maness and Anderson, 2013; Ronget *et al*., 2018; Jones and Ward, 2020).

To address the equivocal nature of studies to date, it is important to consider causative versus correlative links between morphological traits and survival. Increased wing length may be causally related to decreased fledgling mortality because it improves flight ability (Cornell *et al*., 2017; Jones and Ward, 2020), likely facilitating the evasion of predators (Martin, 2015). However, some morphological traits (e.g., body mass) are supposedly correlated with survival because they reflect physiological ‘condition’ or quality (Ardia, 2005; Cuervo *et al*., 2011; Minias, 2015). Yet, empirical evidence linking morphological and physiological traits in juveniles is lacking, especially considering the variability of developmental trajectories between species. For example, many species display mass-overshoot-recession growth profiles where asymptotic mass is attained well before fledging, followed by rapid pre-fledging mass recession (Phillips and Hamer, 1999; Gray and Hamer, 2001; Masello and Quillfeldt, 2002; Phillips *et al*., 2003; Wright *et al*, 2006; Goodpaster and Ritchison, 2014). Furthermore, whilst some morphological traits such as tarsus and wing length generally increase linearly with age, final size at fledging can be influenced by earlier developmental conditions and compensatory growth (Bize *et al*., 2003, 2006; Hegyi and Török, 2007; Criscuolo *et al*., 2008; Krause and Naguib, 2011). In such cases, it is uncertain how varying morphological trajectories interact with a suite of physiological traits (e.g., through developmental trade-offs) that have themselves also been linked to fitness (Thomas *et al*., 2007; Noguera *et al*., 2012; Bowers *et al*., 2014; Cornell *et al*., 2017; Boonekamp *et al*., 2018; Brown *et al*., 2021), especially approaching fledging. Considering fledging represents a key life-stage transition marked by high levels of mortality (Cox *et al*., 2014), investigating relationships among traits at this transition could provide insights into how variable developmental trajectories (cf. absolute size) facilitate carryover effects between life-stages to influence overall fitness.

In this study, we measured developmental trajectories of morphological traits (mass, tarsus, and wing length) approaching independence and final physiological condition (aerobic capacity, energy state, and oxidative status) in the European starling (*Sturnus vulgaris*), a species that displays a mass-overshoot-recession growth profile (Cornell and Williams, 2017). We analyzed changes in morphological traits approaching fledging in relation to physiological traits at fledging, whilst also assessing relationships among traits at fledging. Our objectives were to (1) examine individual variation in the developmental trajectories of morphological traits approaching fledging, exploring patterns that may reflect developmental plasticity (e.g., adaptive mass recession), (2) assess if individual variation of morphological developmental trajectories predict fledgling physiology, consistent with developmental trade-offs and/or preferential resource allocation, and (3) investigate relationships among morphological and physiological traits at fledging, testing the assumption that traits relating to body size are useful metrics of overall quality because they reflect physiological condition.

1.3. Methods

In 2020 and 2021, we measured morphological developmental trajectories approaching fledging and overall morphological and physiological condition at fledging in European starlings at our long-term study side of Davistead Farm, Langley, British Columbia, Canada (49°10'N, 122°50'W). Here, nest boxes are monitored daily from egg-laying until fledging or failure to determine lay date, egg size and clutch size. At 15 days post-hatching, when European starlings typically reach asymptotic mass, two chicks were randomly selected from broods of 4–6 chicks (mean brood size 17 days after hatching = 4) as controls for a separate experiment. These control chicks provided all the data presented within this study.

1.3.1. Field and laboratory methods

Chicks (*n* = 98) at first brood nests (*n* = 49) were weighed (body mass, ± 0.01 g) and measured (tarsus, ± 0.01 mm, wing chord, ± 0.5 mm) 15, 17, and 20 days after hatching between 10:00 and 16:30 to assess morphological development. Also on day 20, one day prior to typical fledging age (>90% of chicks fledge on day 21; Cornell *et al*., 2017), we used heparinized capillary tubes to take 100–500 uL of blood (<10% of total blood volume) from the wing's brachial vein, immediately following initial nest disturbance to minimize handling time. Handling times were calculated for each individual and were defined as the time between initial nest disturbance and the time each chick was weighed (for days 15–20) or blood sampled (day 20). Mass was not related to handling time at any age ($p > 0.22$ in all cases) nor time of day ($p > 0.38$). Additionally, no physiological trait showed any relation to handling time ($p > 0.36$) or time of day ($p > 0.22$). Using these samples (*n* = 89), we assessed fledgling physiological condition by measuring three distinct traits: aerobic capacity (haematocrit and haemoglobin), energy state (plasma triglycerides), and oxidative status (reactive oxygen metabolites (dROMs) and antioxidant titers (OXY)). Nest boxes were checked at day 22 to confirm fledge success; 9 chicks fledged before day 20 measurements and all remaining chicks fledged by day 22. Birds that fledged before day 20 were not blood sampled and therefore not included in any analysis of physiological traits.

Haematocrit (% packed cell volume) was measured (± 0.01 mm) using digital calipers after whole blood was centrifuged for three minutes at 13,000 *g* (Microspin 24; Vulcon Technologies, Grandview, MO, USA). Haemoglobin (g dl^{-1}) was measured using the cyanomethaemoglobin method (Drabkin and Austin, 1932) modified for use with a microplate spectrophotometer (BioTek Powerwave 340; BioTek Instruments, Winooski, VT, USA), measuring 5 µl whole blood diluted in 1.25 ml Drabkin's reagent (D5941; SigmaAldrich Canada, Oakville, Ontario, Canada) at 540 nm absorbance. Plasma triglycerides (mmol l^{-1}) were measured with a colorimetric assay (Sigma-Aldrich Co.) following the manufacturer's guidelines. Reactive oxygen metabolites (mg H_2O_2 dl^{-1}) and total antioxidant titers (µmol HClO ml^{-1}) were measured using dROMs and OXY kits from Diacron International (Grosseto, Tuscany, Italy). Using a single pooled plasma sample, we calculated intra-assay coefficients of variation (CV) by running 10 replicates of this pool on an initial quality control plate (haemoglobin = 0.9%; plasma triglycerides = 2.67%; OXY = 9.92%; dROMs = 4.77%). Each subsequent plate was run alongside the same pool to calculate inter-assay CVs (haemoglobin = 2.77%; plasma triglycerides = 6.06%; OXY = 4.27%; dROMs = 5.98%). Samples were run in duplicates or triplicates; any sample with a CV greater than 10% was rerun. Red blood cells (n = 43) were sent to HealthGene Laboratory (Concord, Ontario, Canada) for sexing by polymerase chain reaction alongside known adult samples for quality control. Sex was only known for one year due a freezer malfunction (during the COVID-19 lockdown) that resulted in the loss of red blood cells from 2020.

1.3.2. Statistical methods

Statistical analyses were performed in RStudio v.4.0.3 (RStudio, *Inc.*, Boston, MA, USA). We tested all traits for normality (Shapiro-wilks test); plasma triglycerides and dROMs data were logarithmically transformed. Tarsus and wing length were not normally distributed but showed no improvement when transformed. We therefore used quantile-quantile plots of studentized residuals for models involving tarsus and wing length to remove points of undue influence which significantly deviated (fell outside 95% confidence bands) from the normal distribution; a maximum of four individuals were removed from any single model. Akaike's information criterion (AIC) was used to select main effect-covariate interaction terms. Interaction terms were incorporated where a lower AIC score supported their inclusion and are reported when significant. In-text values are presented as means ± standard deviation.

We first ran bivariate analyses to independently test the effects of ecological context (brood size and year) and sex on pre-fledging morphological development and fledgling (day 20) morphological and physiological maturity. We created linear mixed-effect models (LMMs) using the change (Δ) in morphological traits (mass or wing length) between ages 15–20 and ecological context and sex as main effects. Nest ID was included as a random factor in all our models. Since chick tarsi were fully developed by 15 days old (mean Δ tarsus length between day 15–20 = 0.01 ± 0.39 mm), displaying no relationship with age (LMM: p = 0.38), we only considered tarsus length at day 20 in our models and did not analyze Δ tarsus length. We used similar LMMs to test for ecological context and sex effects on fledgling developmental maturity, replacing Δ morphological traits with day 20 morphological and physiological trait measurements as main effects. Next, we tested for relationships among morphological and physiological traits using LMMs and controlling for ecological context and sex. Results from models testing for effects of ecological context and sex on development, as well as covariation among morphological and physiological traits, are reported in Appendix A. These models informed covariate selection within our subsequent analyses; we included any significant effects (i.e., $p < 0.05$) as covariates. Where sex was used as a covariate, models were first run without sex as a covariate and using both years of data, then again using only 2021 data to test for covariance by sex.

To explore morphological development prior to fledging, we tested whether morphological traits at day 15 predicted subsequent changes to day 20 by using day 15 mass or wing length and Δ mass or wing length between days 15–20 as main effects. We used LMMs with repeated measures to test for relationships between morphological traits and age (days 15–20), using chick ID nested within nest ID as random factors. Finally, we used two approaches to investigate associations between morphological development and fledgling physiology: (1) LMMs to analyze direct correlations between individual morphological and physiological traits, potentially reflecting mechanistic links between growth and physiology, and (2) Principal component analysis with LMMs to test whether morphological traits at fledging predict overall indices of physiological condition. For the first approach, we tested whether each of the five physiological traits at day 20 were independently correlated with Δ morphological traits (mass and wing length) between ages 15–20 and day 20 measurements (mass, tarsus length, and wing length). For the latter approach, we identified only one principal component (PC1) of physiological condition with an eigenvalue significantly greater than 1 (PC1 eigenvalue = 2.1), which explained 42% of the variance in fledgling physiology. Three of the five physiological traits contributed to the majority of PC1 variance (Figure 1.3A): haemoglobin (loading: 0.54), haematocrit (0.53), and plasma triglycerides (0.46), each of which was significantly positively correlated with PC1 ($p < 0.01$ in all cases). We calculated Principal Component (PC) scores for each individual for PC1 and tested for general associations between individual variation in these PC scores and morphological measurements at day 20, using LMMs with nest ID nested within year as random factors.

1.4. Results

1.4.1. Individual variation in the developmental trajectories of morphological traits

Mass at day 15 was significantly negatively correlated with Δ mass to day 20 (Figure 1.1A; $p < 0.01$, $F_{1,80} = 11.3$) and there was a significant mass*brood size interaction ($p < 0.01$, $F_{1,79} = 7.28$), mass*year ($p = 0.02$, $F_{1,78} = 5.81$), year*brood size ($p = 0.04$, $F_{1,77} = 4.49$) and mass*brood size*year interaction ($p = 0.04$, $F_{1,77} = 4.44$). The brood size*year interaction revealed a positive, but nonsignificant, relationship between brood size and mass loss in 2020 ($p = 0.24$, $F_{1,22} = 1.48$) but a negative relationship in 2021 ($p = 0.09$, $F_{1,15} = 3.34$). In 2020, brood sizes of four displayed the greatest Δ mass between days 15–20 (-7.27 ± 5.14 g), whereas in 2021 Δ mass was greatest in brood sizes of six (-3.68 ± 2.5 g). Despite a significant mass*year interaction, the negative relationship between mass at day 15 and Δ mass between days 15–20 was significant in both 2020 ($p < 0.01$, $F_{1,42} = 13.9$) and 2021 ($p = 0.02$, $F_{1,31} = 5.69$). Day 15 wing length was also significantly negatively correlated with subsequent Δ wing length to day 20 (Figure 1.1B; $p < 0.01$, $F_{1,86} = 63.5$).

Mass was significantly negatively related to age between days 15–20 (Figure 1.1C; $p < 0.01$, $F_{1,187} = 155$), with chicks losing 3.98 ± 3.48 g between days 15–20. However, wing length was significantly positively related to age (Figure 1.1D; $p < 0.01$, $F_{1,185} = 10195$), growing 20 ± 1.84 mm between days 15–20. To test if the negative correlations between size at day 15 and subsequent changes to day 20 reduced individual variation in morphological traits approaching fledging, we performed post-hoc F-tests to compare the variance of morphological traits at day 15 versus day 20. There was no significant difference in the variance of mass between days 15–20 in 2020 ($p = 0.55$, $F_{1,45} = 1.2$) or 2021 ($p = 0.81$, $F_{1,42} = 0.93$). However, variance in wing length at day 20 was significantly lower than at day 15 ($p = 0.04$, $F_{1,87} = 1.55$).

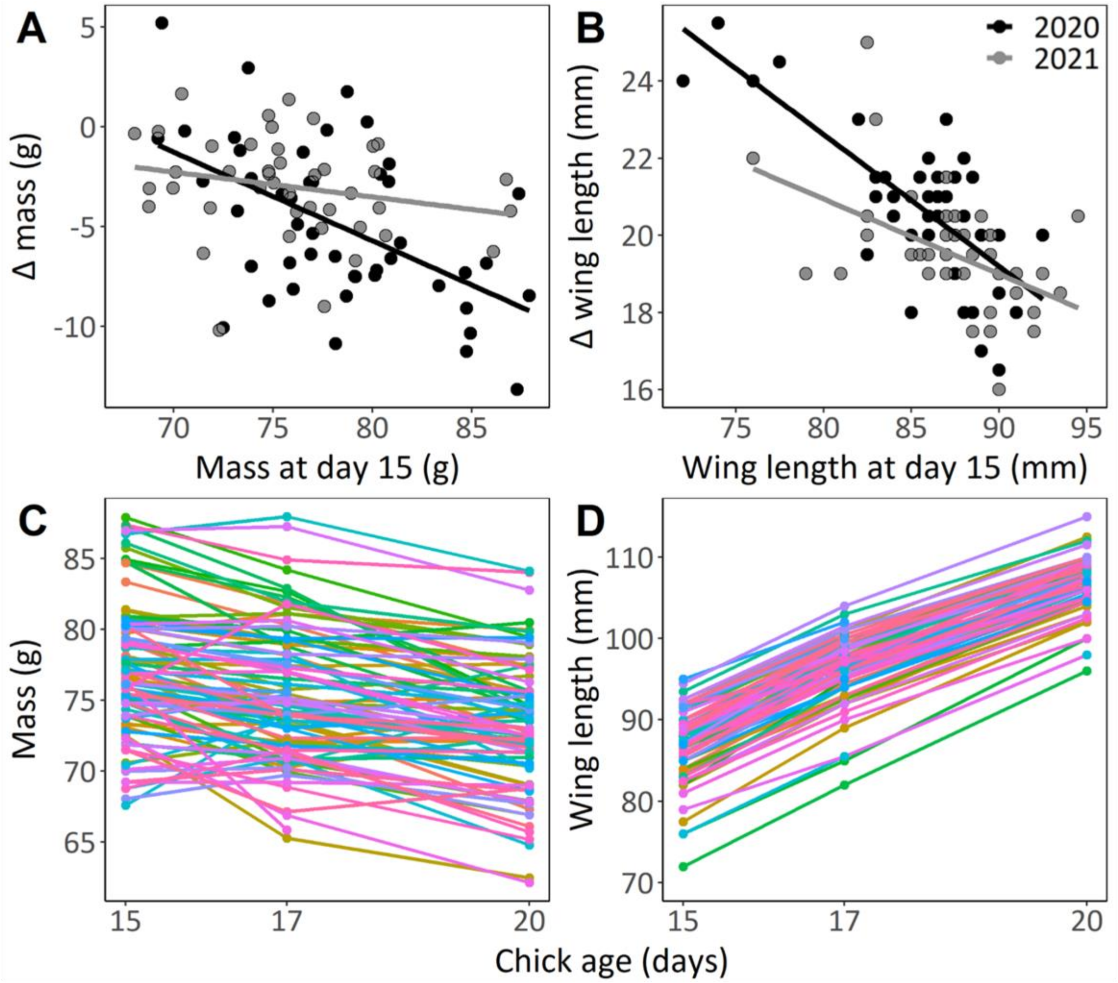

Figure 1.1 **Individual variation of morphological development approaching fledging in the European starling (*Sturnus vulgaris*) across two years. Mass (A) and wing length (B) 15 days after hatching in relation to subsequent changes (∆) in these traits between days 15–20. Mass (C) and wing length (D) on days 15, 17, and 20. All relationships were significant and are reported in "Results". For covariation of morphological development by year, see Table A.1.**

1.4.2. Pre-fledging morphological growth trajectories in relation to fledgling physiology

Individual variation in ∆ mass between days 15–20 was significantly positively related to day 20 plasma triglycerides ($p < 0.01$, $F_{1,75} = 7.36$) but showed no relation to haematocrit ($p = 0.72$, $F_{1,79} = 0.13$), haemoglobin ($p = 0.36$, $F_{1,79} = 0.86$), dROMs ($p =$

0.13, $F_{1,80} = 2.3$), or OXY ($p = 0.16$, $F_{1,78} = 1.99$). Individual variation in Δ wing length between days 15–20 was significantly negatively related to day 20 haematocrit (Figure 1.2A; $p = 0.03$, $F_{1,76} = 5.11$) and haemoglobin (Figure 1.2B; $p < 0.01$, $F_{1,66} = 8.64$), but showed no relationship to plasma triglycerides ($p = 0.07$, $F_{1,79} = 3.37$), dROMs ($p = 0.73$, $F_{1,81} = 0.12$), or OXY ($p = 0.66$, $F_{1,80} = 0.2$).

1.4.3. Relationships between morphological and physiological traits at fledging

There was a positive correlation between day 20 mass and plasma triglycerides that approached significance ($p = 0.05$, $F_{1,74} = 3.83$), however this relationship was only significant in 2021 when we could control for sex ($p = 0.04$, $F_{1,32} = 4.72$). Haematocrit, haemoglobin, dROMs and OXY were all independent of day 20 mass ($p > 0.09$ in all cases). However, mass was significantly positively related to PC1 (Figure 1.3B; $p = 0.04$, $F_{1,84} = 4.19$). Tarsus and wing length at day 20 were unrelated to all physiological traits ($p > 0.3$ in all cases), as well as PC1 (Figure 1.3C-D; $p > 0.76$ in all cases).

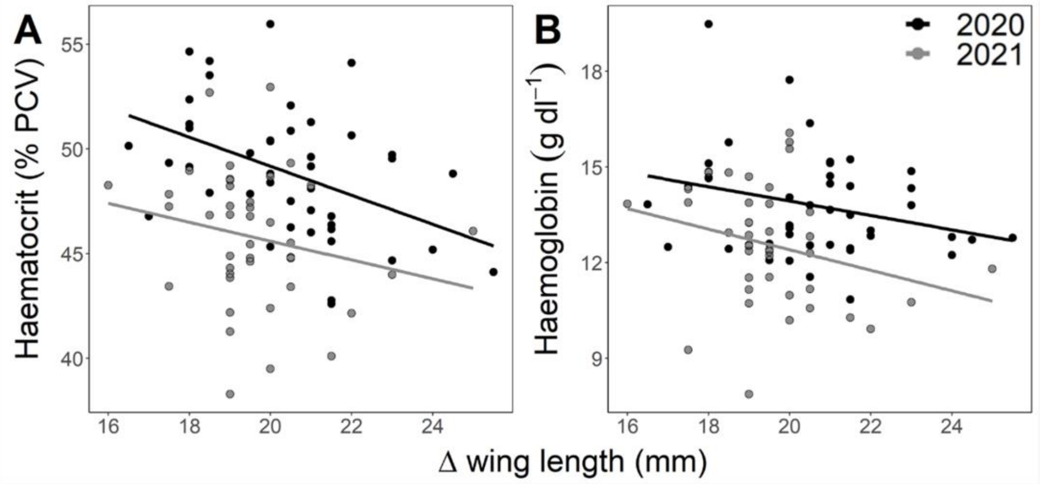

Figure 1.2 **Covariation between wing growth approaching fledging and fledgling aerobic capacity. Change (Δ) in wing length between days 15–20 (A, B) in relation to day 20 haematocrit (A) and haemoglobin (B). Both relationships were significant (see "Results"). For covariation of physiological traits by year, see Table A.1.**

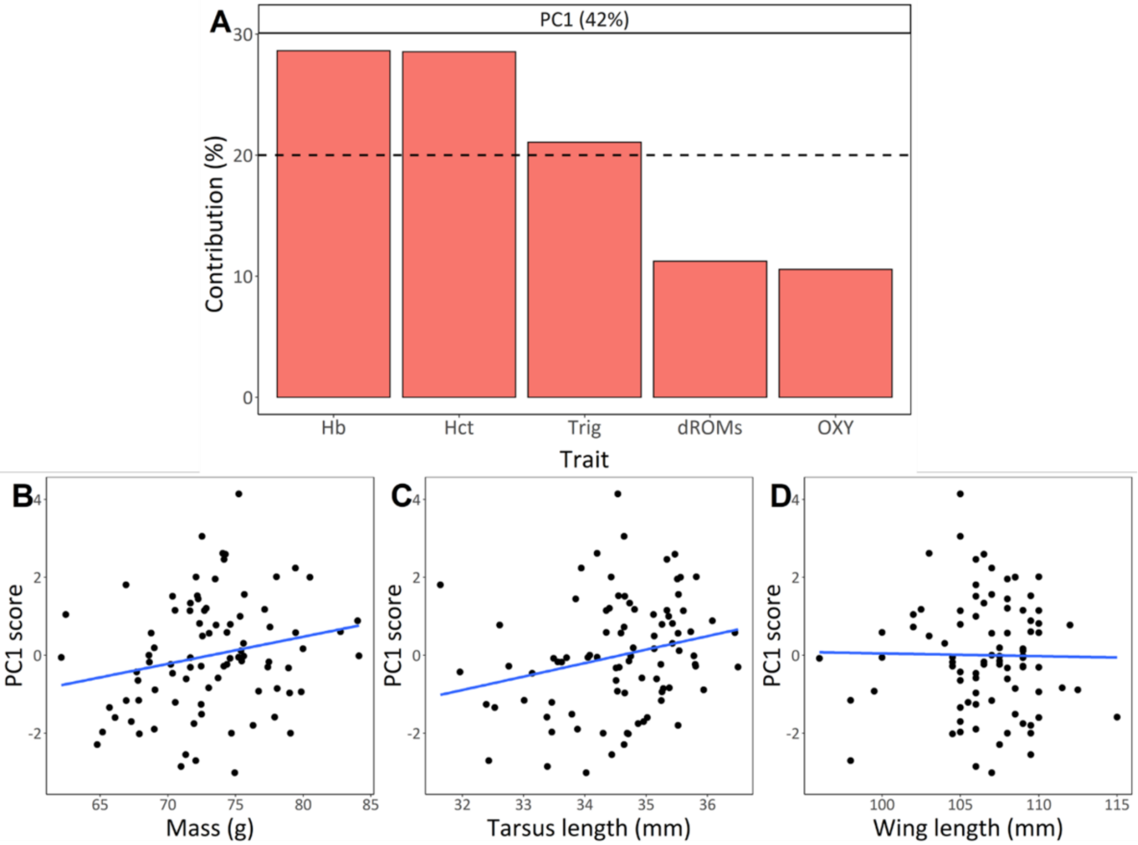

Figure 1.3 **Principal components of fledgling physiology versus morphological traits. Contributed variance of physiological traits to principal component one (PC1) of fledgling physiology (A). Individual scores for PC1 in relation to day 20 mass (B), tarsus length (C), and wing length (D). Dashed line in (A) is the expected average contributed variance by each trait to the principal component. Only the relationship in (B) was significant (see “Results”). Hb, haemoglobin; Hct, haematocrit; Trig, plasma triglycerides; dROMs, reactive oxygen metabolites; OXY, antioxidant titers.**

1.5. Discussion

We investigated covariation between morphological and physiological development approaching fledging in juvenile European starlings, focusing on the changes of morphological traits just prior to fledging and correlating these to physiological traits at fledging. Individual variation in *changes* in morphological traits were consistent with marked development plasticity and were better predictors of individual physiological traits at fledging than absolute fledgling size. Nestling mass and wing length at day 15 strongly predicted subsequent growth to day 20, suggesting adaptive mass recession and compensatory wing growth respectively, which coincided with a significant decrease in variance of wing length over the same period. Such plasticity may function to ensure beneficial carryover effects into the post-fledging period, as flexibility of these traits may promote juvenile flight ability (e.g., optimizing wing load; Wright *et al*., 2006; Goodpaster and Ritchison, 2014; Cornell *et al*., 2017), a strong predictor of survival between bird species (Martin *et al*., 2018; Jones and Ward, 2020). However, our data suggest physiological costs for flexible growth, potentially associated with resource allocation decisions, as increased mass recession and wing growth approaching fledging were associated with decreased energy state and aerobic capacity. This covariation could lead to similar fitness-related morphological traits at fledging (e.g., wing length) but variable and hidden physiological costs, paradoxically reducing the potential for such traits to predict post-fledging survival (Boonekamp *et al*., 2017). Furthermore, we identified one principal component of fledgling physiology, explaining 42% of physiological variance across individuals that represents the best metric of overall physiological condition. Fledgling mass was positively correlated with PC1, however wing and tarsus length at fledging were independent of all physiological traits measured, as well as PC1.This suggests that metrics of both growth and body size may reflect physiological condition, but these relationships may vary between morphological traits and depend on whether physiological traits are considered individually (as in many studies) or collectively.

1.5.1. Individual variation in the developmental trajectories of morphological traits

The development of morphological traits approaching fledging has previously been reported in this species, revealing significant wing growth but mass recession (Cornell and Williams, 2017), as seen here. However, by exploring these trajectories further, we have shown that morphological trait values at 15 days after hatching strongly predict subsequent growth to fledging at day 20. Pre-fledging mass recession was highly variable among individuals and was seemingly dictated by asymptotic mass; heavier individuals at day 15 generally lost more mass to day 20, whereas some individuals showed no change in mass or even an increase in mass between days 15–20. This may also explain the variation of mass trajectories by year (Appendix A, Table A.1), as chicks in 2020 were significantly heavier on day 15 but lost more mass to day 20. Consequently, chick mass on day 20 was similar between years (Appendix A, Table A.1), despite very different pre-fledging trajectories. Thus, pre-fledging mass recession does not follow a hardwired developmental trajectory in European starlings but is instead a flexible process that can be adjusted based on the rate of mass gain prior to asymptotic mass, which likely varies by ecological context. This is consistent with experimental studies which have demonstrated facultative mass recession in swifts (Wright *et al*., 2006; Goodpaster and Ritchison, 2014), but are somewhat contradicted by similar studies in other species with mass-overshoot-recession growth profiles where nestlings showed no adjustment to an experimental increase in their pre-fledging mass (Mitchell, 2018; Moeller and Ritchison, 2019).

In addition to developmental plasticity of mass trajectories approaching fledging, nestlings displayed flexible wing growth between days 15–20 in relation to their wing length at day 15. Nestlings with smaller wings at day 15 generally grew their wings at a much greater rate to day 20 than individuals with longer wings. This pattern is consistent with compensatory growth, in which animals exhibit faster than usual growth rates following a period of resource deprivation (Wilson and Osbourn, 1960). Consequently, variance in nestling wing length decreased as wing lengths converged towards a mean value at fledging. Fledging with underdeveloped wings likely incurs higher post-fledging mortality (Martin *et al*., 2018; Jones and Ward, 2020). However, instead of delaying fledging to facilitate catch-up growth (Lepczyk and Karasov, 2000; Bize *et al*., 2003; Aldredge, 2016), European starlings seemingly prioritize accelerated, compensatory growth approaching independence, perhaps to facilitate synchronous fledging within and/or across broods. This supports the idea that compensatory growth evolves where there are constraints on development time (Yearsley *et al*., 2004; Dmitriew, 2011).

Whilst compensatory growth is typically defined by a return to more favourable conditions following a period of fasting (Hector and Nakagawa, 2012), we do not have the data to support this mechanism. Whilst we have several years of data suggesting there is no change in provisioning rate by nestling age in European starlings (Allen, unpublished data), we cannot rule out that changes in food allocation by parents prior to fledging (perhaps induced by increased begging in smaller nestlings; Rydén and Bengtsson, 1980) is the mechanism for compensatory growth in this species. However, we suggest that an alternative mechanism for compensatory growth could be preferential resource allocation towards (or developmental trade-offs favouring) key fitness traits approaching life-stage transitions (see below), that need not be facilitated by realimentation (Boujard *et al*., 2000; Dmitriew *et al*., 2007).

Decreased individual variation of wing length approaching fledging is consistent with developmental canalization, whereby traits with larger fitness effects (like wing length: Morrison *et al*., 2009; Cornell *et al*., 2017; Martin *et al*, 2018; Jones and Ward, 2020) exhibit less phenotypic variation in response to genetic and environmental changes (Waddington, 1942; Flatt, 2005; Boonekamp *et al*., 2017, 2018). Indeed, wing growth was seemingly robust to contextual perturbations, developing independently of brood size, sex, and year (Appendix A, Table A.1). As such, trajectories of structural traits may appear to be developmentally hardwired when observed as an average across a population and/or species. However, by exploring variable growth between individuals, we have demonstrated a high level of flexibility of wing development which suggests certain traits may be more plastic than previously thought in some species. Such plasticity does not negate canalized wing development though, as variable growth rates resulting from preferential resource allocation (reviewed in Mainwaring and Hartley, 2012) may in fact promote an ecological robustness of certain traits (Liefting *et al*., 2015).

1.5.2. Pre-fledging morphological growth trajectories in relation to fledgling physiology

There was covariation between morphological and physiological traits approaching fledging, suggesting potential energetic costs of pre-fledging mass recession and aerobic costs of compensatory wing growth. Decreased plasma triglyceride levels in relation to greater mass recession is consistent with a mechanism of lipid catabolism underpinning pre-fledging mass recession (Thomas *et al*., 1993; Ricklefs and Schew, 1994; Riou and Hamer, 2010), potentially contradicting Lack's (1968) energy reserves hypothesis suggesting fitness advantages for fatter fledglings (but see below). Furthermore, there is some support to suggest nestlings are not losing mass solely by fasting, as this may lead to a reduction in metabolites and protein oxidation (Houser *et al*., 2021), whilst we found no relationship between mass recession and indices of oxidative status here. This is consistent with previous studies showing no change in provisioning rate or begging intensity during pre-fledging mass recession (Goodpaster and Ritchison, 2014; Moeller and Ritchison, 2019).

Flexibility of wing development was also associated with physiological costs, as compensatory wing growth seemingly resulted in decreased fledgling aerobic capacity. Similar results have been reported in laboratory studies showing nestlings directing investment towards structural growth at the expense of organ development in response to food shortages (Moe *et al*., 2004; Kilpack and Karasov, 2012). Studies that have explored physiology in relation to compensatory growth largely focus on oxidative and metabolic costs (Alonso-Alvarez *et al*., 2007; Criscuolo *et al*., 2008; de Block and Stoks, 2008; Smith *et al*., 2016; Constantini *et al*, 2018; Burraco *et al*, 2020; Janssens and Stoks, 2020), yet few have tested immediate trade-offs between such growth trajectories and other metrics of physiological quality, such as aerobic capacity and energy state. These immediate trade-offs are potentially more significant determinants of recruitment than deferred costs, as they are more pertinent to the immediate post-fledging period and the high rates of juvenile mortality that characterize it (Cox *et al*, 2014). Whilst many studies have shown oxidative costs associated with growth (reviewed in Smith *et al*., 2016), we found no evidence to support that here. Perhaps it is plausible that individuals transitioning to independence with reduced aerobic capacity, a trait linked to post-fledging flight ability and survival (Thomas *et al*., 2007; Bowers *et al*., 2014; Jones and Ward, 2020; Brown *et al*, 2021), experience increased oxidative stress later in life as the physiological immaturity of these traits fail to meet performance demands.

1.5.3. Relationships between morphological and physiological traits at fledging

Much work has explored whether individual variation in morphological traits reflect physiological quality but mainly in mature, adult animals (Ardia, 2005; Fair *et al*., 2007; Amat *et al*., 2009; Lill *et al*., 2013; Minias, 2015). However, in nestlings where both suites of traits are still developing, it is uncertain how morphological and physiological condition are related. Despite this, morphological traits alone are often used to assess individual quality at fledging, often under the assumption that variation in these traits reflect physiological ‘quality’. We found evidence to suggest body mass, but not structural size, at independence reflects physiological condition. Despite marked mass recession prior to fledging, fledgling mass was nonetheless positively associated with

PC1. The majority of the variance in PC1 was explained by metrics of aerobic capacity, which have been linked to flight ability in European starlings previously (Cornell *et al*., 2017). Thus, the contribution of aerobic traits towards flight ability may enable individuals of better physiological quality to maintain more mass prior to fledging, potentially to experience adaptive benefits of improved energy state associated with increased mass shown here. Conversely, the lack of an association between structural traits and physiological condition may be driven by plasticity of growth trajectories prior to fledging, which reflected individual variation of fledgling aerobic capacity. Whilst this plasticity may facilitate the attainment of normal fledgling size, it potentially leads to greater physiological variation as observed previously (Cornell and Williams, 2017). The resulting decreased variance in key fitness traits (e.g., wing length) approaching fledging potentially uncouples any correlations among developing structural and physiological traits.

The ecological robustness and developmental prioritization of wing growth approaching fledging supports recent studies suggesting wing size is a morphological trait with strong links to post-fledging survival between species (Martins *et al*., 2018; Jones and Ward, 2020), providing further evidence that this trait is canalized (Boonekamp *et al*., 2017). However, such canalization may actually reduce the potential for wing length to predict post-fledging survival within species, as observed in Jones and Ward (2020). This may be due to weak links with developmental conditions (Boonekamp *et al*., 2017) and/or trade-offs that facilitate canalized morphological developmental trajectories at the expense of physiological condition (Moe *et al*., 2004; Kilpack and Karasov, 2012; this study). Thus, body mass and wing length may be variously linked to rates of juvenile mortality through indirect versus direct associations with fitness; mass may generally reflect physiological traits that are correlated with survival, whereas wing length may directly determine survival by reflecting juvenile flight ability, but inconsistencies at predicting survival within species could be related to developmental trade-offs between growth and physiology, as suggested here.

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1.7. References

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Chapter 2.

Decoupling of body size, physiological state, and subsequent performance in a developmentally manipulated songbird

2.1. Chapter abstract

It is generally assumed that larger juveniles are more physiologically mature, hence their overall condition and subsequent performance are higher. Some taxa face extreme workload transitions during development (e.g., nest departure in birds), which may select for non-linear growth profiles that decouple relationships between body size and physiological state, making the links between metrics of development (e.g., body mass) and juvenile performance uncertain. We manipulated perceived mass in European starlings approaching fledging using 4.0 g weighted backpacks, measuring subsequent growth trajectories (mass, wing length) and physiological state (aerobic capacity, energy state, oxidative status) to test whether body size and physiology are coupled during a non-linear developmental phase (pre-fledging mass recession). Nanotag radio transmitters were then used to track post-fledging performance (activity, activity-slope, dispersal). Weighted nestlings had greater pre-fledging mass recession and marginally reduced wing growth when compared to controls. Consequently, weighted birds had lower body mass at fledging, while also displaying markers of oxidative stress (increased reactive oxygen metabolites), yet aerobic capacity and energy state were equal between treatment groups. Previously weighted birds showed no correlation between fledgling state (body size, physiology) and subsequent performance. However, control fledglings that had decreased mass recession and greater wing growth prior to fledging dispersed earlier, while longer wings at fledging were associated with more rapid increases in post-fledging activity (activity-slope). In addition, control fledglings with lower haemoglobin also dispersed earlier, while increased reactive oxygen metabolites were associated with decreased activity levels. Body mass and energy state were unrelated to any metric of post-fledging performance. Our data suggest mass-independent development of some physiological traits in species with non-linear growth profiles, potentially with a context-dependent oxidative cost, and

that physiology may predict some measures of post-fledging performance not captured by body size.

2.2. Introduction

Extreme workload transitions may pose a significant challenge to an individual's fitness if they are not matched by compensatory preparations; this is well-known and intuitive in the sporting world where intense activity can result in poor performance and injury in individuals that have not trained appropriately (Armstrong, 1984; Appell *et al*., 1992). Through exercise, athletes achieve a suite of morphological (body size) and physiological trait values that improve performance and alleviate costs of increased activity. Workload transitions are also an integral part of some free-living animals' life histories, such that pre-emptive energetic investment or divestment in specific traits may also be advantageous. Long-distance migration, for example, may be preceded by fat deposition (Bairlein, 2002), 'exercise organ' hypertrophy (Piersma, 1998), 'nutritional organ' atrophy (Piersma *et al*., 1999), erythropoiesis (Krause *et al*., 2016), and changes in muscular biochemistry (Banerjee and Chaturvedi, 2016). The extent to which such plasticity is exhibited prior to migration may be correlated with an individual's quality (*sensu* Wilson and Nussey, 2010) and ultimately determine survival through the workload transition from a non-migratory to migratory state. Another key life-history transition associated with changes in workload occurs at morphological maturation and independence in many taxa. Here, mortality in juvenile animals is likely determined by development prior to independence (Ronget *et al*., 2018). This may be particularly true in birds, as the period after fledging (nest departure) is marked by high rates of juvenile mortality, resulting in a bottleneck for recruitment (Cox *et al*., 2014).

In altricial birds, fledging represents an extreme workload transition where chicks go from a completely sedentary, inactive lifestyle in the nest to an active lifestyle post-fledging where flight ability is critical for survival (Lind *et al*., 2010; Martin *et al*., 2018; Jones and Ward, 2020). Developmental plasticity in juvenile birds may be an evolutionary consequence of this transition, as conflict in the adaptive function of traits between inactive and active life stages selects for flexible growth preceding fledging (reviewed in Mainwaring and Hartley, 2012), likely prioritizing morphological traits related to flight and producing beneficial carryover effects to improve post-fledging performance (Jones and Ward, 2020). For example, large fat reserves may buffer against variable conditions during development (Lack, 1968; Ricklefs, 1990; Phillips and Hamer, 1999; de Zwaan *et al*., 2020) but reduce flight performance after fledging (Witter and Cuthill, 1993). Thus, nestlings in some species display pre-fledging mass recession where nestlings achieve, or often exceed, adult mass relatively early in development, only to lose a significant portion of that mass prior to fledging (Phillips and Hamer, 1999; Gray and Hamer, 2001; Masello and Quillfeldt, 2002; Phillips *et al*., 2003). Despite this, body mass is typically used as a metric of juvenile quality as it supposedly reflects physiological state or maturity (Ardia, 2005; Cuervo *et al*., 2011). However, whether non-linear growth profiles, like mass overshoot-recession, affect the putative coupling of morphological and physiological development remains uncertain (but see Simmons and Lill, 2006). Moreover, the importance of physiological state at fledging for post-fledging performance is critically understudied. Morphological traits at fledging might explain interspecific rates of juvenile mortality but their utility for predicting individual variation in survival within species is equivocal (Magrath, 1991; Schwagmeyer and Mock, 2008; Maness and Anderson, 2013; Ronget *et al*., 2018; Jones and Ward, 2020). Investigating the links between non-linear developmental trajectories, physiological state, and post-fledging performance might therefore provide insight into what determines individual quality at this key life-stage transition.

Here, we experimentally tested the coupling of morphological and physiological traits during a period of non-linear development by manipulating perceived mass in juvenile European starlings (*Sturnus vulgaris*) at the beginning of pre-fledging mass recession. We fitted nestlings with 4.0 g (approx. 5% peak body mass) weighted backpacks six days before fledging and measured subsequent morphological developmental trajectories (mass recession, wing growth) and fledgling physiology (aerobic capacity, energy state, and oxidative status) in relation to weight treatment (WT). Fledglings were then fitted with VHF radio transmitters (Nanotags) to track the performance of experimental and control birds up to six weeks post-fledging. Radio transmitter data was used to obtain three metrics of post-fledging performance: activity, activity-slope, and dispersal, all of which have been linked to juvenile survival (Rotics *et al*., 2021). We predicted that weighted nestlings would compensate for an increase in their perceived mass and facultatively lose more mass than controls (e.g., as shown in swifts: Wright *et al*., 2006; Goodpaster and Ritchison, 2014); increased mass recession in WT chicks would therefore lead to decreased body mass at fledging. In relation to physiological state, we predicted that weighted nestlings would fledge with: (i) decreased energy state (plasma triglycerides) resulting from facultative metabolization of fat reserves as a mechanism for mass recession (Ricklefs, 1968; Martins, 1997; Allen *et al*., 2022), (ii) reduced aerobic capacity as experimentally decreased body mass would be linked with lower haematocrit (Cuervo *et al*., 2011; Allen *et al*., 2022) and haemoglobin (Allen *et al*., 2022), and (iii) decreased oxidative status (increased plasma reactive oxygen metabolites, decreased antioxidant capacity), as accelerated mass recession would increase metabolic activity and therefore exacerbate the production of free radicals (Monaghan *et al*., 2009), potentially reflecting oxidative stress. Finally, we predicted that weighted nestlings would exhibit decreased post-fledging performance (decreased activity and activity-slope, earlier dispersal) resulting from negative effects of treatment on wing-loading and physiological state, which have been linked to fledgling flight ability (Cornell *et al*., 2017) and recruitment (Bowers *et al*., 2014), respectively.

2.3. Materials and methods

We measured morphological developmental trajectories of European starlings approaching fledging and overall morphological and physiological state at fledging in three consecutive years (2020–2022), tracking post-fledging activity in two of those years (2021–2022), in addition to validating our experimental design using a short field-experiment in 2023 (see below). Fieldwork was conducted at our long-term study site of Davistead Farm, Langley, British Columbia, Canada (49°10'N, 122°50'W), where 150 nestboxes are monitored daily from egg-laying to fledging or failure. All research was conducted under Simon Fraser University animal care permit 1018 B-96 and 1172B-96 following guidelines of the Canadian Council of Animal Care (CCAC), and a banding permit from Environment Canada (permit number 10646 to TDW).

2.3.1. Experimental design

Chicks (*n* = 244) from first broods (*n* = 61) were weighed (body mass, ± 0.01 g) and measured (wing chord, ± 0.5 mm) at 15 days post-hatching, when asymptotic mass is typically attained in European starlings, and again on days 17 and 20 (one day prior to mean fledging age). At day 15, we randomly selected four chicks from brood sizes of 4–6, two of which were fitted with 4.0 g lead-weighted backpacks (approx. 5% peak body mass) using a leg-loop harness (see Rappole and Tipton, 1991), with the remaining two chicks receiving no treatment and acting as controls. Weights that fell off treatment chicks (*n* = 23) between days 15–20 were excluded from our dataset, leaving *n* = 221 chicks for analysis. To validate that our results were due to effects of the additional weight itself on mass recession and not, for example, the effect of the harness (e.g., restricted movement, biased feeding by parents), in 2023 unweighted backpacks (0.86 ± 0.04 g) were put on *n* = 22 chicks, with matched controls. Since chick tarsi are fully developed by 15 days old, exhibiting no growth (mean change in tarsus between days 15–20 = -0.01 ± 0.03 mm) or significant relationship with age (*p* = 0.19), we did not consider tarsus length in this study. At day 20, chick weights were removed and 100-500 µL of blood (<10% of total blood volume) was taken from the brachial vein using heparinized capillary tubes. Handling times (time between initial nest disturbance and weighing/blood sampling) were recorded for all individuals (mean handling time = 3.95 ± 0.11 min). There was no difference of handling time between treatment groups (*p* = 0.15). Overall, handling time was significantly negatively correlated with dROMs (*p* = 0.02) while time of day (between 09:45 and 16:20) was significantly negatively correlated with haematocrit (*p* < 0.01). Consequently, handling time and time of day were controlled for in models analysing dROMs and haematocrit, respectively. No other trait was related to handling time or time of day (*p* > 0.06 in all cases).

We assayed blood samples for five physiological traits, representing three metrics of physiological state: aerobic capacity (haematocrit and haemoglobin), energy state (plasma triglycerides), and oxidative status (OXY and dROMs). Standard assay methods were used to analyze blood samples (see Allen *et al*., 2022); we used a single pooled plasma sample to calculate intra- and inter-assay coefficients of variation (CV). This pool was run on an initial quality control plate with 10 replicates (intra-assay CV: haemoglobin = 0.9%; plasma triglycerides = 2.67%; OXY = 9.92%; dROMs = 4.77%) and alongside each subsequent plate (inter-assay CV: haemoglobin = 2.27%; plasma triglycerides = 8.61%; OXY = 4.07%; dROMs = 11.2%). Samples were assayed in duplicates or triplicates and any sample with a CV > 10% was re-assayed. Red blood cells from 2021 and 2022 were sexed by polymerase chain reaction after being sent to HealthGene Laboratory (Concord, Ontario, Canada) with known-sex adult samples for validation. Sexing data were not available in 2020 as red blood cell samples were lost due to a freezer malfunction during the COVID-19 lockdown.

2.3.2. Automated radio telemetry

At day 20, treatment and control chicks were fitted with digitally coded nanotag radio transmitters (NTQB-4-2, Lotek Wireless Inc., Newmarket, Ontario, Canada) to track fledgling activity between nest departure and dispersal or death (WT: *n* = 45; control: *n* = 44). Four automated radio telemetry 'towers' (ART) were erected at typical foraging areas around the field site; each tower comprised an automated receiver unit (Lotek Wireless Inc.) and four five-element Yagi antennas at 90° angles covering 360°. Every antenna scanned for nearby (approx. 1km; validation in Serota and Williams, 2019) radio transmitters every 8 seconds for 49 days from when the first radio transmitters were deployed. The receiver produces a power output for a detected radio transmitter that corresponds to the signal strength (distance from tower and angle to receiving antenna); this power value is relatively consistent between detections when a radio transmitter is not moving but varies greatly when it is moving (Ward *et al*., 2014; Greives *et al*., 2015). We therefore used the change (Δ) in power between successive detections as a measure of activity. The Δ power of incubating females was used in Serota and Williams (2019) to test for baseline variations in signal strength that occur in non-moving radio transmitters to determine a threshold of Δ power 10, above which corresponded to an 'active' detection. We validated this finding here by analyzing chick activity preceding fledging and at night, as well as radio transmitters placed in stationary locations about the field site (Appendix B, Figure B.4 and B.5). Thus, we adopted the same threshold of Δ power 10 in this study as in Serota and Williams (2019). Using the number of 'active' detections relative to total detections (i.e., active detections/total detections × 100), and including variation in detection frequency between tags as a covariate, we calculated marginal means of daily activity (%) for each fledgling. Radio transmitter validation suggested that obtaining fine-scale activity data was less likely at distances greater than 700m from the detecting receiver, which is accompanied by a decrease in number of detections (also see Crewe *et al*., 2019). We therefore filtered our dataset to only include activity data from towers where >100 detections had been made within a day. Our analyses were additionally restricted to daylight hours as determined by civil twilight. Three metrics for post-fledging performance (Appendix B, Figure B.3) were calculated from daily activity data: (1) average post-fledging activity (%), calculated as the overall mean of activity from all days prior to dispersal or the last day of observation, (2) activity-slope, calculated as the slope coefficient of daily activity between fledging and dispersal or the last day of observation, and (3) day of dispersal, as the final day with >100 detections at a single tower. We assumed mortality where a radio transmitter produced 0% activity for five or more consecutive days.

2.3.3. Statistical analysis

Statistical analyses were performed in RStudio v4.2.0 (RStudio, *Inc*., Boston, MA, USA). Shapiro-Wilks tests were used to test all traits for normality; haemoglobin, plasma triglycerides and dROMs were logarithmically transformed. Δ wing length, wing length, activity and activity-slope were not normally distributed but showed no improvement when transformed. Consequently, we utilized quantile-quantile plots of studentized residuals to remove points of undue influence which significantly deviated from the normal distribution (fell outside 95% confidence bands); this was done on a per-model basis for all models that analysed Δ wing length, wing length, activity, and activity-slope data. We built models including sex where preliminary analyses indicated traits and/or performance varied significantly by sex (Appendix B, Table B.1). Sex interaction terms were used where a lower AIC score supported their inclusion (cf. sex as a covariate) and are reported when significant (see Appendix B for example handling of sex data). Brood size was constrained in our experimental design and preliminary analyses suggested no interactions. Our models therefore included brood size along with year and nest ID as random factors. In-text values are presented as means ± s.e.

Treatment effects on morphological trajectories were tested using linear mixed-effects models (LMMs) that included age and mass or wing length as main effects as well as an age × treatment (WT, control) interaction; chick ID nested within nest ID were included as random factors to account for repeated measures. If the age × treatment interaction was significant, we analysed the two measurements periods (i.e., days 15–17 and 17–20) by calculating marginal means and using paired contrasts (emmeans) to compare differences in growth between groups separately within each period. Using the same models but adjusted pair contrasts, we also tested for treatment effects on final fledgling mass and wing length. To test for treatment effects on fledgling physiology, we calculated marginal means for WT and control chicks from LMMs, with each physiological trait in a separate model and treatment as a main effect, and used paired contrasts. To test the effects of the unweighted backpacks on mass recession, we pooled data from all years and used a LMM with change in mass and treatment (WT, unweighted backpack, control) as main effects, including year as a covariate and nest ID as a random factor. We then calculated marginal means of mass recession and tested for differences between treatments using paired contrasts.

We analysed two overlapping subsets of post-fledging performance data: (1) chicks that showed no evidence of mortality, and (2) chicks that showed no evidence of mortality and had more than five days of activity data. The former was used to analyse day of dispersal, as accurate activity data was not necessary to determine when a radio transmitter was no longer being consistently detected. However, we constrained our analysis of average post-fledging activity and activity-slope to the latter data set, as too few days of activity data would affect the accuracy of both metrics. We tested for direct consequences of WT on post-fledging performance using paired contrasts of marginal means calculated from LMMs with treatment and activity, activity-slope, or day of dispersal as main effects. In addition, we tested for correlations between chick development (morphological trajectories, as well as final fledging morphological and physiological trait values) and post-fledging performance while including WT as an interaction term (e.g., Activity ~ Mass*WT). If the interaction was significant, we separated our analyses into WT and controls to independently test for correlations between chick development and post-fledging activity within each group.

2.4. Results

2.4.1. Validation of weight treatment

In the validation analysis, there was no difference in mass loss between days 15–17 (when the treatment effect occurs in the main experiment; see below) when comparing control chicks (-1.56 ± 0.29 g) and those with unweighted backpacks (-2.50 ± 0.70 g; $p = 0.19$, $t_{1,243} = 1.31$). In contrast, mass loss was significantly greater in chicks with weighted backpacks (-4.63 ± 0.32 g) when compared to both chicks with unweighted backpacks and controls ($p < 0.01$ in both cases).

2.4.2. Effects of weight treatment on development

In the main weighted backpack experiment (2020–2022), mass was significantly negatively related to age between days 15–20 (Figure 2.1A; p < 0.01, $F_{2,435} = 356$), confirming mass recession. Trajectories differed between treatment groups and varied by age, signified by a significant age × WT interaction ($p < 0.01$, $F_{2,435} = 30.5$); WT chicks demonstrated significantly greater mass recession than controls between days 15–17 ($p < 0.01$, $t_{1, 435} = -7.09$), though not between days 17–20 ($p = 0.5$, $t_{1,435} = 0.68$). WT chicks lost 6.73 ± 0.30 g between days 15–20, compared to 4.12 ± 0.28 g in controls, yet two thirds of that mass (4.58 ± 0.29 g) was lost in the first two days following treatment. By comparison, mass recession in controls followed a linear trajectory with chicks losing 1.69 ± 0.28 g between days 15–17 and 2.43 ± 0.28 g between days 17–20. WT chicks therefore seemed to partially compensate for the 4.0 g added weight, losing 2.61 ± 0.41 g more body mass than controls between days 15–20, and consequently had lower body mass on day 20 (Table 2.1).

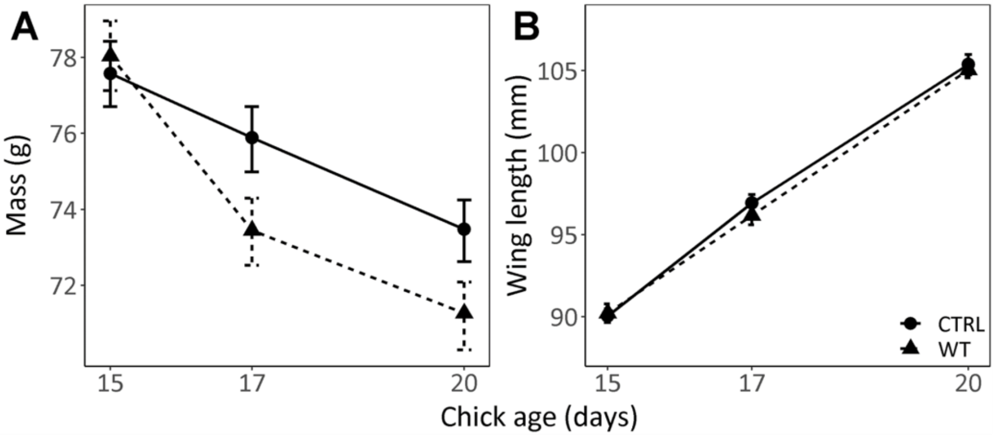
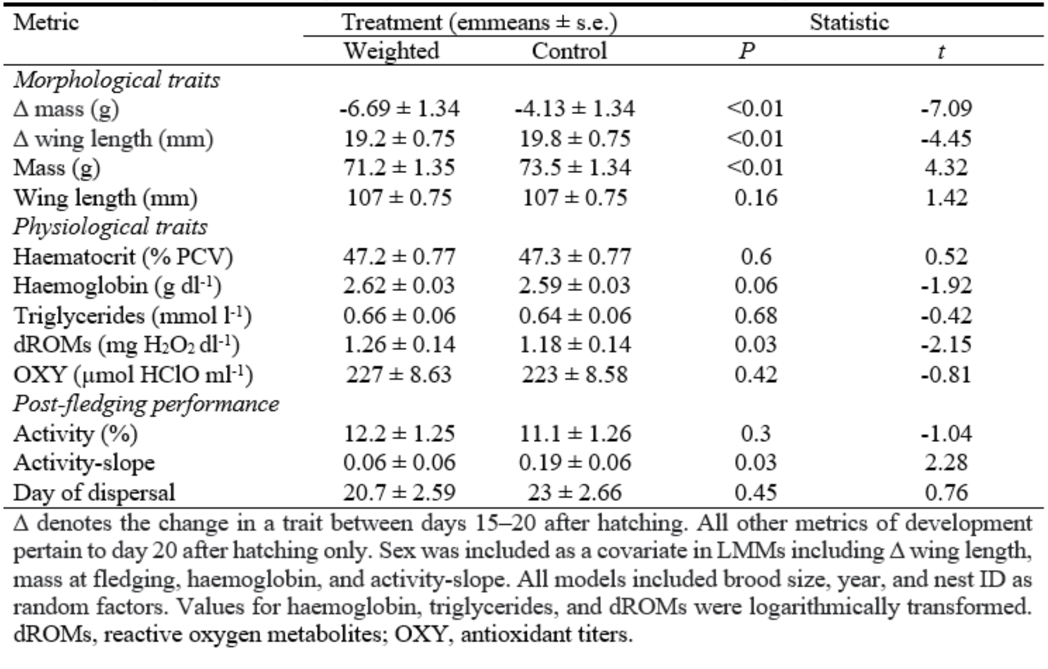


Figure 2.1 **Morphological traits approaching fledging in control and weight-treated European starling (*Sturnus vulgaris*) nestlings. Chick age (A, B) in relation to mass (A) and wing length (B). Line-type and point-shape correspond to control (CTRL) and weight-treated (WT) chicks. Changes in mass and wing length were significantly different between groups, in addition to a WT × age interaction; trait changes between groups were only significantly different between ages 15–17 (see "Results").**

Wing length was significantly positively related to age (Figure 2.1B; $p < 0.01$, $F_{2,415} = 22959$), and, again, trajectories differed by treatment group and varied by age, indicated by a significant age × WT interaction ($p < 0.01$, $F_{2,415} = 11.6$). Wing growth in WT chicks was significantly less than controls in the first two days after treatment ($p < 0.01$, $t_{1,415} = -3.79$), while there was no difference in growth between days 17–20 ($p = 0.49$, $t_{1,415} = -0.68$). However, differences in wing growth between days 15–17 were small, with WT chicks' wings growing 8.46 ± 0.13 mm compared to 9.14 ± 0.13 mm in controls and wing length at day 20 was not significantly different between treatments (Table 2.1). Data from 2021–2022 also showed sex-based variation in the consequences of treatment for wing growth, as there was a significant age × WT × sex interaction ($p = 0.04$, $F_{2,240} = 3.22$). While there was no significant sex difference in wing growth resulting from treatment between ages 15–17 ($p = 0.33$, $t_{1,240} = -1.61$), wing growth between days 17–20 resulting from treatment was significantly different by sex ($p = 0.04$, $t_{1,240} = 2.5$). In female control chicks, day 17–20 wing growth was 2% less than WT chicks ($p = 0.52$, $t_{1,60} = -0.65$), whereas in male control chicks day 17–20 wing

Table 2.1 **Estimated marginal means (emmeans) and paired contrast results of variation in morphological and physiological development, as well as post-fledging performance, by treatment in European starlings (*Sturnus vulgaris*). Chicks were measured in British Columbia, Canada, between 2020–2022, starting at 15 days after hatching and ending on day 20, one day prior to typical fledging age. Post-fledging performance pertains to data from 2021–2022 only.**



Metric	Treatment (emmeans ± s.e.)		Statistic	
	Weighted	Control	P	t
Morphological traits				
Δ mass (g)	-6.69 ± 1.34	-4.13 ± 1.34	<0.01	-7.09
Δ wing length (mm)	19.2 ± 0.75	19.8 ± 0.75	<0.01	-4.45
Mass (g)	71.2 ± 1.35	73.5 ± 1.34	<0.01	4.32
Wing length (mm)	107 ± 0.75	107 ± 0.75	0.16	1.42
Physiological traits				
Haematocrit (% PCV)	47.2 ± 0.77	47.3 ± 0.77	0.6	0.52
Haemoglobin (g dl^{-1})	2.62 ± 0.03	2.59 ± 0.03	0.06	-1.92
Triglycerides (mmol l^{-1})	0.66 ± 0.06	0.64 ± 0.06	0.68	-0.42
dROMs (mg H_2O_2 dl^{-1})	1.26 ± 0.14	1.18 ± 0.14	0.03	-2.15
OXY (μmol HClO ml^{-1})	227 ± 8.63	223 ± 8.58	0.42	-0.81
Post-fledging performance				
Activity (%)	12.2 ± 1.25	11.1 ± 1.26	0.3	-1.04
Activity-slope	0.06 ± 0.06	0.19 ± 0.06	0.03	2.28
Day of dispersal	20.7 ± 2.59	23 ± 2.66	0.45	0.76

Δ denotes the change in a trait between days 15–20 after hatching. All other metrics of development pertain to day 20 after hatching only. Sex was included as a covariate in LMMs including Δ wing length, mass at fledging, haemoglobin, and activity-slope. All models included brood size, year, and nest ID as random factors. Values for haemoglobin, triglycerides, and dROMs were logarithmically transformed. dROMs, reactive oxygen metabolites; OXY, antioxidant titers.

growth was 6% greater than WT chicks ($p = 0.03$, $t_{1,51} = 2.25$). However, wing growth in WT chicks between days 17–20 did not vary by sex ($p = 0.23$, $t_{1,58} = -1.22$). In addition, there was no significant WT × sex interaction with wing length at day 20 ($F_{1,236} = 0.83$, $p = 0.36$). Since treatment effects were observed for both wing length and mass, we performed a post-hoc analysis testing for covariation between changes in these traits across all chicks; increased mass recession was significantly associated with decreased wing growth (Appendix B, Figure B.8A; $p < 0.01$, $F_{1,210} = 12.6$).

dROMs at fledging were greater in WT chicks, but haematocrit, haemoglobin, triglycerides, and OXY did not vary by treatment (Table 2.1).

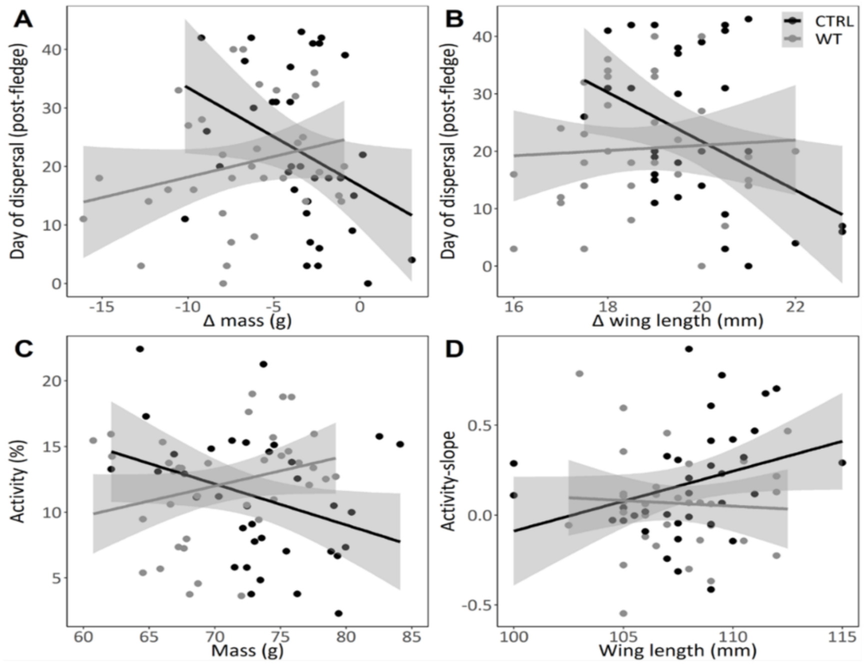


Figure 2.2 Morphological traits in relation to metrics of post-fledging performance. Δ mass vs day of dispersal (A), Δ wing length vs day of dispersal (B), mass at fledging vs activity (C), and wing length at fledging vs activity-slope (D). Lines correspond to control (CTRL) and weight-treated (WT) chicks. All WT interactions were significant, however only in (A,B,D) were there significant relationships within controls. No relationship in WT chicks was significant (see "Results"). Δ denotes the change in a trait between days 15–20 after hatching.

2.4.3. Developmental and experimental consequences for post-fledging performance

Average post-fledging activity across all chicks was 11.65 ± 1.26 %. Average activity and day of dispersal were unaffected by treatment (Table 2.1), though control chicks showed a greater rate of increase in activity after fledging than WT chicks, suggesting WT chicks were more active just after fledging. We performed a post-hoc analysis on immediate post-fledging activity by treatment group, testing whether the difference in activity-slope, yet similarity in overall activity, was driven by contrasts in activity immediately after fledging. In the first 10 days after fledging, WT chicks (14.5 ± 1.66 %) were significantly more active than controls ($p = 0.01$, $t_{1,34} = -2.64$), but showed little subsequent increase in activity between fledging and dispersal. Conversely, controls were less active immediately after fledging (12.4 ± 1.71 %) but increased their activity between fledging and dispersal (Appendix B, Figure B.6).

There was a significant Δ mass × WT and Δ wing length × WT interaction with day of dispersal (Figure 2.2A,B; $p \leq 0.01$ in both cases). Neither Δ mass nor Δ wing length was related to day of dispersal in WT chicks ($p > 0.08$ in both cases), yet both

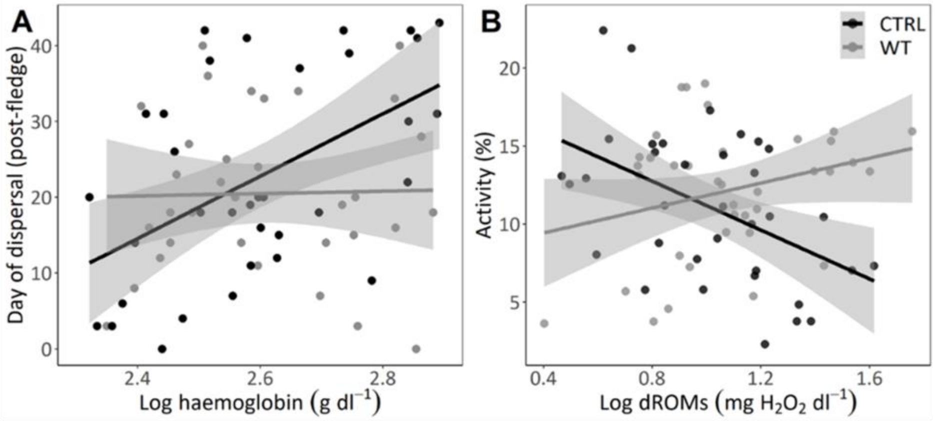


Figure 2.3 Physiological traits in relation to metrics of post-fledging performance. Log haemoglobin vs day of dispersal (A), and log dROMs vs activity (B). Lines correspond to control (CTRL) and weight-treated (WT) chicks. Relationships in controls only, as well as WT interactions, were significant in both (see "Results"). dROMs, reactive oxygen metabolites.

were significantly negatively related to day of dispersal in controls ($p \leq 0.01$ in both cases). Additionally, there was a fledgling mass × WT interaction with activity (Figure 2.2C; $p < 0.01$, $F_{1,57} = 8.02$) but neither WT nor control fledgling mass showed significant correlations with activity when analysed separately ($p > 0.08$ in both cases). However, there was a fledgling wing length × WT interaction with activity-slope (Figure 2.2D; $p = 0.04$, $F_{1,47} = 4.5$); wing length and activity-slope were significantly positively correlated in controls ($p = 0.03$, $F_{1,29} = 5.04$) but not in WT chicks ($p = 0.29$, $F_{1,31} = 1.16$).

There was a significant haemoglobin × WT interaction with day of dispersal (Figure 2.3A; p = 0.03, $F_{1,52} = 4.95$). Haemoglobin and day of dispersal were positively correlated in controls ($p < 0.01$, $F_{1,29} = 17.9$) but not in WT chicks ($p = 0.24$, $F_{1,26} = 1.47$). There was also a significant dROMs × WT interaction with average activity (Figure 2.3B; $p < 0.01$, $F_{1,63} = 11.8$); dROMs and activity were significantly negatively correlated in controls ($p < 0.01$, $F_{1,32} = 9.53$) but not in WT chicks ($p = 0.11$, $F_{1,32} = 2.66$). No other relationships between physiological traits and post-fledging performance were significant, nor were there any significant WT interactions ($p > 0.06$ in all cases).

2.5. Discussion

We investigated developmental responses of nestling European starlings to a manipulation of their perceived mass, testing for: (i) plasticity of non-linear developmental trajectories approaching fledging, (ii) coupling of morphological and physiological traits during a period of non-linear development, and (iii) consequences for post-fledging performance up to six weeks after nest departure. Experimental nestlings compensated for ~64% of the added weight on average, displaying significantly greater mass recession than controls, whereas wing growth was significantly, but marginally (3%), reduced. Differences in morphological trajectories between treatments suggest facultative mass recession in European starlings (Wright *et al*., 2006; Goodpaster and Ritchison, 2014), with a potential cost of the increased mass loss on wing growth (Wright *et al*., 2006). Despite marked differences in mass at fledging between treatments, most physiological traits were unaffected, suggesting mass-overshoot recession (and potentially other non-linear growth profiles) can decouple body size from physiological state, highlighting the need to consider species-specific developmental modes when assessing juvenile quality. However, increased dROMs in WT chicks suggest a context-dependent oxidative cost of maintaining developmental trajectories of several traits related to flight ability (e.g., wing length, haematocrit, haemoglobin; Cornell *et al*., 2017) while experiencing greater declines in body mass (Cornell and Williams, 2017). Variation in morphological and physiological states did not predict differences in post-fledging performance between treatments. WT birds were more active than control birds immediately after fledging but control birds subsequently increased their activity more rapidly approaching dispersal. No morphological or physiological trait predicted performance in WT birds, whereas certain morphological and physiological traits were significantly related to post-fledging performance in controls. Changes in mass and wing length approaching fledging were negatively correlated with dispersal timing, whereas wing length at fledging was positively associated with the rate of change in activity between fledging and dispersal. In addition, haemoglobin was positively correlated with dispersal timing, while dROMs was negatively associated with average activity, providing the first evidence of relationships between these metrics of post-fledging performance and physiological state.

2.5.1. Effects of weight treatment on developmental trajectories of morphological traits

Our experimental design sought to increase perceived body mass in European starlings to test whether chicks would facultatively adjust their mass in response (as in Wright *et al*, 2006; Goodpaster and Ritchison, 2014; Moeller and Ritchison, 2019). An alternative explanation for differences in mass loss between treatments could relate to effects of the harness and/or backpack per se, rather than the added weight, e.g., the fitted weight could have restricted movement and negatively affected sibling competition, while there could have been differential treatment by parents during feeding. However, the similarity in mass recession between chicks with unweighted backpacks and controls suggest that the weight itself, and not differences in food acquisition relating to the harness, was important for the observed differences in mass recession. The added 4.0 g weight might have carried energetic costs when nestlings were active, increasing mass loss in treatment chicks (cf. a facultative response). However, this is unlikely since nestling European starlings are highly inactive in the approach to fledging (Allen, 2023; unpublished behavioural data from nest cameras) and comparable plasma triglyceride levels between treatments suggest similar energy states at fledging. Finally, differences in morphological trajectories in our study could alternatively reflect a general response to stress of the backpack treatment. To explore this, we measured plasma corticosterone in a random subset of n = 13 samples from control (n = 7) and WT (n = 6) individuals at day 17, immediately following the period of increased mass recession in WT chicks. There was no difference in corticosterone concentrations between control and WT chicks (Appendix B, Figure B.10A), suggesting differences in mass recession between groups did not simply reflect a generic response to stress.

We therefore suggest that European starlings demonstrated facultative mass recession due to a perceived increase in body mass associated with experimental weight treatment. This has been reported in swifts (Wright *et al*., 2006; Goodpaster and Ritchison, 2014) but not tree swallows (Moeller and Ritchison, 2019) and American kestrels (Mitchell, 2018). The reason for these conflicting results is unclear but appears to be independent of the duration of nestling development: European starlings showed highly flexible body mass trajectories in a 21-day nestling period that is similar to tree swallows which show no flexibility of mass recession (Moeller and Ritchison, 2019). In addition, our data suggest a potential cost for mass recession in terms of reduced wing growth, as also found in swifts (Wright *et al*., 2006). An adaptive response to an increase in perceived wing-load might be to increase wing growth, yet the adaptive mass response to treatment coincided with a ‘nonadaptive’, albeit small, decrease in wing growth over the same period. Based on correlations between plasma triglycerides and changes in mass and wing length (Appendix B, Figure B.8), our data suggest that increased mass recession is associated with decreased energy available for wing growth. So, the partial compensation for the added weight might reflect trade-offs between optimizing wing-loading (by increasing mass recession) and preserving energy state (Allen *et al*., 2022). Moreover, while the effects of treatment on mass recession were consistent between the sexes, the putative effects on wing growth varied between males and females. Control male nestlings accelerated their wing growth in the final three days prior to fledging, resulting in significant sex differences of Δ wing length between days 17–20, with a potential cost to haemoglobin (Appendix B, Figure B.9). However, treatment seemingly compromised males’ ability to accelerate wing growth prior to fledging, as there was no sex difference in Δ wing length between days 17–20 in WT chicks, suggesting sex-biased costs of increased mass recession.

2.5.2. Effects of weight treatment on fledgling physiology

While weight treatment resulted in clear effects on mass recession, there was little effect of treatment on physiological state, especially for ‘developmental’ traits such as haematocrit and haemoglobin, which both show marked sub-adult maturity at fledging (Cornell *et al*., 2017). This suggests mass-independent, and potentially hardwired, development of some physiological traits in species with non-linear developmental profiles. However, previous work suggests hardwired growth and physiological trajectories may produce an oxidative cost under poor developmental conditions (Cornell and Williams, 2017), and this is supported here. For example, European starling chicks previously showed increased dROMs in low quality years and second broods, but little variation in key fitness-related traits (wing length, aerobic capacity; Cornell and Williams, 2017). In WT chicks, development of the same fitness-related traits (wing length, aerobic capacity) was largely unperturbed by treatment, while dROMs increased (as in Cornell and Williams, 2017). Since previous work has shown that dROMs and mass recession are not correlated in European starlings (Allen *et al*., 2022), perhaps the increase in dROMs in WT chicks was a cost of maintaining most developmental trajectories during a period of experimentally increased mass loss (potentially comparable to poor developmental conditions). Additionally, OXY was equal between groups, suggesting the increase in dROMs caused an imbalance between reactive oxygen species and antioxidant defences, indicative of decreased oxidative status following treatment (Metcalfe and Alonso-Alvarez, 2010; Costantini, 2016). Thus, while hardwired developmental trajectories may ensure certain fitness-related traits are robust to environmental perturbations (Waddington, 1942), this might be traded-off against oxidative status when developmental conditions are poor, which may subsequently influence post-fledging performance (Noguera *et al*., 2012).

We predicted that both aerobic capacity and energy state would co-vary with changes in mass, although the lack of variation in plasma triglycerides between treatment groups was particularly surprising. Previous work has shown strong correlations between Δ mass and plasma triglycerides, which would be consistent with fat catabolism as a mechanism for mass recession (Allen *et al*., 2022). Our results here suggest that changes in mass and plasma triglycerides are simply correlated and the metabolization of energy stores is not a mechanism for mass recession. Instead, mass recession could be driven by water loss from tissue maturation in nestlings approaching fledging (Ricklefs, 1968; Phillips and Hamer, 1999), although no study has yet experimentally tested this.

2.5.3. Developmental and experimental consequences for post-fledging performance

Variation in morphological and physiological state between treatment groups did not explain different patterns of activity in WT chicks. The weight treatment not only decoupled putative links between body mass and physiology, but it also decoupled relationships between fledgling state and post-fledging performance. No morphological or physiological trait was correlated with performance in WT chicks whereas relationships were apparent in control chicks (see below). If radio tags themselves were additional stressors, then stress effects on post-fledging performance should have been equivalent between groups, as both control and WT chicks were fitted with radio tags prior to fledging. Instead, perhaps a combination of decreased oxidative status and unoptimized wing-loading at fledging made post-fledging performance in WT chicks unpredictable by our developmental metrics. For example, increased dROMs could have negatively impacted muscle function in a way not captured by our metrics of physiological state (Steinbacher and Eckl, 2015), potentially influencing fledgling flight ability (Biewener, 2011). Variation in anti-oxidant defences against increased dROMs could have then caused differing developmental consequences for WT chicks, producing diverse treatment effects on post-fledging performance. Previous work has also highlighted how a potential function of pre-fledging mass recession is optimizing wing-loading at fledging (Wright *et al*., 2006; Goodpaster and Ritchison, 2014). By increasing mass recession, we likely produced a mis-match between body mass and wing area, two morphological traits instrumental for flight ability (Witter and Cuthill, 1993; Cornell *et al*., 2017; Jones and Ward, 2020), that could have significantly influenced fledgling behaviour. This, in addition to the potential developmental consequences of decreased oxidative status, could have overridden typical predictors of post-fledging performance in WT chicks, resulting in the observed treatment interactions.

In controls, both morphological and physiological traits predicted metrics of post-fledging activity as well as dispersal. However, we found no relationship between fledgling mass and post-fledging performance, despite previous work linking body mass to physiological state (Ardia, 2005; Cuervo *et al*., 2011; Forsman *et al*., 2010; Allen *et al*.,

2022) and subsequent survival (reviewed in Ronget *et al*., 2018). The lack of an association between body mass and juvenile performance here, alongside evidence of mass-independent development of physiological traits, highlights the potential unreliability of only using body mass as a metric of individual quality in species with non-linear growth profiles. Yet, another metric of body size, wing length, was positively correlated with activity-slope, supporting previous work indicating wing length is a key fitness-related trait for juvenile birds (Martin *et al*., 2018; Jones and Ward, 2020). Activity-slope has been suggested as a potential measure of behavioural development in juveniles (Rotics *et al*., 2021), where a greater slope represents increasing skill acquisition across the post-fledging period, perhaps related to foraging/self-feeding ability. Greater flight ability at fledging, resulting from increased wing length (Cornell *et al*., 2017; Jones and Ward, 2020), may therefore accelerate behavioural development in fledglings, potentially increasing survival. In contrast, Δ mass and wing length prior to fledging were unrelated to activity but instead predicted dispersal timing. Previous work in European starlings showed that the largest individuals at asymptotic mass on day 15 subsequently displayed the greatest mass recession and least wing growth to day 20, whereas the smallest nestlings at day 15 demonstrated compensatory wing growth and mass acquisition (Allen *et al*., 2022). Our data therefore suggest that compensatory growth trajectories are associated with early dispersal, whereas individuals that approached morphological maturity earlier in development disperse later. Individuals that suffered constrained development are more likely to have low quality parents (Coulson and Porter, 1985; Arnold *et al*., 2004), which may also provide limited post-fledging care, potentially encouraging early dispersal in their offspring. This could lead to inadequate pre-dispersal experience and would agree with previous work linking a shorter post-fledging period and reduced post-fledging parental care to lower survival (Naef-Daenzer and Grüebler, 2016; Rotics *et al*., 2021).

Importantly, in our study, indicators of reduced physiological state were also correlated with poor post-fledging performance in controls. Low haemoglobin was associated with early dispersal, agreeing with a previous study suggesting trade-offs between compensatory wing growth and haemoglobin (Allen *et al*., 2022), as both predicted the same metric of performance here. In addition, control chicks with greater dROMs, indicative of decreased oxidative status and physiological state, demonstrated lower activity levels between fledging and dispersal. Cell damage resulting from increased free radical production could limit activity levels, since muscular atrophy, a known consequence of decreased oxidative status (Steinbacher and Eckl, 2015), likely decreases fledgling flight ability (Biewener, 2011). Reduced post-fledging activity is associated with decreased foraging time and first-year survival in white storks (Rotics *et al*., 2021), suggesting fitness consequences for increased dROMs, supported by previous work showing oxidative stress decreases recruitment in European shags (Noguera *et al*., 2012). Thus, the relationship between oxidative status and activity reported here potentially supports the proposition that activity levels are correlated with survival because they reflect individual quality (Rotics *et al*., 2021). Together, our data demonstrate plasticity of mass trajectories approaching the ‘workload transition’ at fledging, in addition to mass-independent development of some physiological traits, potentially with a context-dependent oxidative cost, and highlight the importance of considering physiological state when predicting juvenile performance.

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2.7. References

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Chapter 3.

Variation in oxidative status, but not structural or physiological development, associated with changing ontogenetic environments

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3.1. Chapter abstract

Despite the potential for temporally-dependent relationships between trait values and fitness (e.g., as juveniles approach life-stage transitions such as fledging), how developmental stage affects canalization (a measure of robustness to environmental variation) of morphological and physiological traits is rarely considered. To test the sensitivity of morphological and physiological traits to environmental variation in two developmental stages, we manipulated brood size at hatch in European starlings (*Sturnus vulgaris*) and cross-fostered chicks between enlarged and reduced broods approaching fledging. We measured body size (mass, tarsus, wing length) and physiological state (aerobic capacity, oxidative status) at asymptotic mass on day 15, then cross-fostered chicks between 'high' and 'low' quality environments and assessed the same traits again on day 20, after five days of pre-fledging mass recession. Chicks in reduced broods were heavier at asymptotic mass and had lower reactive oxygen metabolites than enlarged broods, while structural size, aerobic capacity, and antioxidant capacity were unaffected by experimental brood size. The observed canalization of structural and physiological traits during early development was maintained after cross-fostering, during late development. However, in contrast to early development, antioxidant capacity approaching fledging appeared sensitive to environmental conditions, as trajectories varied by cross-fostering treatment. Elevated reactive oxygen metabolites observed after early development in enlarged brood chicks were maintained after cross-fostering, suggesting canalized development in low-quality environments could produce oxidative costs that carry over between life-stages, even when conditions improve. These data reveal trait-specific relationships between environmental conditions

and development and highlight how natal environment effects may vary by developmental stage.

3.2. Introduction

An organisms' natal or developmental environment is known to have long-term fitness consequences (Stearns, 1992; Lindström, 1999; Metcalfe and Monaghan, 2001). Variation in parental effort (Lock *et al*., 2004; Klug and Bonsall, 2014), sibling competition (Ricklefs, 1982; Royle *et al*., 1999; Gil *et al*., 2008), temperature (Jobling, 1997; Van der Have and De Jong, 1996; Weeks *et al*., 2022), predation risk (Lima and Dill, 1990; Abrams and Rowe, 1996), and parasitism (Marcogliese, 2004; Sanchez *et al*., 2018) can all influence patterns of growth, which in turn may have carry-over effects on adult life-history. Species with indeterminate growth are known to make trade-off decisions between reproduction and growth based on developmental conditions experienced as a juvenile (Taborsky, 2006). Conversely, environmental conditions may influence the juvenile phenotype at maturity in species with determinate growth (Abrams *et al.,* 1996; Wells *et al*., 2006), after which many trait values become fixed (De Kogel, 1997; Saino *et al*., 2018). Ontogenetic growth therefore represents a crucial life-history stage that ultimately determines some metrics of the adult phenotype, such as structural size, which are intimately related to fitness (Schmidt-Nielsen, 1984; Ronget *et al*., 2018). These fitness-related traits are expectedly under strong selection for developmental prioritization during this period. Yet, this selection pressure may promote an insensitivity of some traits to developmental conditions (Waddington, 1942; Flatt, 2005; Boonekamp *et al.*, 2017), contradicting the expected relationships between environment and ontogenetic growth. For example, preferential resource allocation has been documented in several species where individuals seemingly maintain structural growth trajectories at the expense of physiological state in response to food restriction (Moe *et al.,* 2004; Killpack and Karasov, 2012). Some species may have therefore evolved behavioural or organismal 'strategies' to promote a developmental robustness of certain fitness-related traits to environmental perturbations.

Preferential resource allocation has been proposed as one potential mechanism of canalization, a measure of developmental (in)sensitivity to environmental variation; traits that strongly determine fitness are predicted to demonstrate greater canalization. For example, wing length in birds has been shown to influence first flight ability and mortality of fledglings in several species (Cornell *et al*., 2017; Martin *et al*., 2018; Jones and Ward, 2020). Consistent with canalization, wing covert fault bars in jackdaws (*Coloeus monedula*) varied little in response to environmental variation (brood size) and poorly reflected developmental conditions when compared to tail covert fault bars (Boonekamp *et al*., 2017). Yet, wing covert fault bars better predicted survival when compared to tail covert fault bars. Canalization of wing growth therefore meant that neither wing nor tail coverts linked developmental conditions to juvenile survival. Ecological studies, however, seldom consider canalization when assessing variation in putative fitness biomarkers or individual quality (*sensu* Wilson and Nussey, 2010) in response to environmental variation. In addition, the degree to which physiological traits are canalized has been virtually unexplored (but see Boonekamp *et al*., 2018), despite their importance for interpreting the potential costs and/or trade-offs associated with canalization (e.g., Moe et al., 2004; Killpack and Karasov, 2012). Finally, few studies have considered how trait variability might be influenced by developmental stage (but see Kok et al., 2019) and approaching life-stage transitions. Boonekamp *et al.,* (2018) predicted that the amount of variability around an ‘optimal trait value’ would be dependent upon the fitness costs associated with deviation from that value. However, the costs of such deviations likely vary depending upon the developmental stage, potential resulting in a temporal influence on trait canalization (e.g., De Zwaan *et al*., 2020). For example, it is unlikely that wing length directly affects mortality in sedentary nestlings. Prioritization of wing development may therefore be biased towards the later stages of nestling growth when flight-related traits become more relevant for fitness. Indeed, individual variation of wing length in European starling nestlings decreases in the final five days prior to fledging, as small chicks seemingly display late-stage catch-up growth (Allen *et al*., 2022). This could suggest that wing length earlier in the nestling period might be more sensitive to environmental conditions, while wing length at fledging appears to be canalized due to limited individual variation and insensitivity to some metrics of ecological context (Cornell and Williams, 2017). While this has yet to be experimentally tested, understanding how juveniles respond to environmental variability during ontogenetic growth could offer valuable perspective into organismal responses to climate change (Svensson *et al*., 2020) and factors influencing individual quality.

In this study, we investigated the effects of the ontogenetic environment on European starling (*Sturnus vulgaris*) development by manipulating brood size at hatch and subsequently cross-fostering chicks in the approach to fledging. European starlings reach asymptotic mass at day 15 post-hatch, after which they demonstrate pre-fledging mass recession to fledging age (21 days post-hatch). Therefore, our experimental design not only facilitates a comparison of development and its costs between low- and high-quality natal environments, but also independently tests whether two different growth phases, up to asymptotic mass on day 15 (‘early’ development) versus during pre-fledging mass recession (‘late’ development), vary in their sensitivity to environmental conditions (e.g., Simon *et al*., 2005). Broods of six (enlarged) and two (reduced) were artificially created on hatch day. We then measured body size (body mass, tarsus, and wing length), aerobic capacity (hematocrit, hemoglobin), and oxidative status (reactive oxygen metabolites, dROMS; antioxidant capacity, OXY) at 15 days after hatching to determine the effects of environmental (brood size) variation on early development to asymptotic mass. Both aerobic capacity and oxidative status have been linked with fitness (Noguera *et al*., 2012; Bowers *et* al., 2014), however aerobic capacity was measured as a marker of physiological development, due its positive association with age and sub-adult maturity at fledging (Cornell *et al*., 2017; Cornell and Williams, 2017). Conversely, oxidative status was used to assess potential costs associated with variable growth, as it has been previously linked to developmental rate (Metcalfe and Alonso-Alvarez, 2010; Smith *et al*., 2016) and possibly canalization (Cornell and Williams, 2017). At the end of the early developmental phase on day 15, two chicks from an enlarged brood were then switched with two chicks from a reduced brood to test the effects of ‘new’ environmental conditions on late development during the phase of pre-fledging mass recession. Body size was then measured on day 17 and 20, and physiological state (aerobic capacity, oxidative status) was also assessed again on day 20, one day prior to fledging, to compare trait canalization between growth phases.

Our predictions were based on a ‘traditional’ view of developmental biology, assuming significant trait variability in response to marked environmental variation. By producing enlarged and reduced broods at hatch, we predicted that chicks in the reduced brood would be fed more frequently, resulting in larger body size and improved physiological state at the end of the early developmental phase. Specifically, we expected reduced broods to exhibit greater physiological maturity of ‘developmental’ traits (increased hemoglobin, hematocrit) and increased oxidative status (decreased reactive oxygen metabolites, increased antioxidant capacity) due to reduced developmental stress (e.g., lower sibling competition, greater provisioning). After cross-fostering and during the late developmental phase, we predicted that chicks switched from an enlarged brood to a reduced brood would accelerate structural development (catch-up growth) and physiological maturation to compensate for poor conditions experienced during early development. Chicks transferred to more favourable developmental conditions (reduced brood) would display greater wing growth and increased physiological development (hemoglobin, hematocrit) relative to chicks in the enlarged brood. We also predicted that chicks transferred to the enlarged brood (from a reduced brood) would display greater mass recession during late development due to increased asymptotic mass on day 15. Furthermore, we expected the same chicks to show decreased oxidative status (increased reactive oxygen metabolites, decreased antioxidant capacity) in response to a decline in developmental conditions (enlarged brood), relative to chicks transferred to the reduced brood.

3.3. Materials and methods

Fieldwork was completed between April and June of 2022 at our long-term study site of Davistead Farm, Langley, British Columbia, Canada (49°10'N, 122°50'W). Here, 150 nestboxes are checked daily during egg-laying (March-April) and subsequently monitored from hatching until fledging or failure (April-June). European starlings are synchronous breeders (80% of nests are laid within 4.8 days; Williams *et al.,* 2015) and show a high degree of hatching synchrony (96% of clutches hatch within 2 days; Gibson and Williams, 2017). Females typically lay clutches of 4–6 eggs, incubating alone for 10–11 days while both parents provision chicks between hatching and 21 days post-hatch, when chicks fledge. Experimental protocols were approved prior to the field season by Simon Fraser University Animal Care Committee (protocol no. 1172B-96-23) and banding was permitted by Environment Canada (permit no. 10646 to TDW).

3.3.1. Experimental design

Upon clutch competition, initial clutch sizes (mean CS = 5.00 ± 0.86 eggs) were recorded, after which all experimental nests (*n* = 20) were left with four eggs to incubate until hatch (natural laying sequence 1–4 in all cases). After the four eggs in an experimental nest had hatched, two chicks were removed at random and placed into another experimental nest that shared the same hatch day, creating one brood of six chicks (enlarged) and one brood of two (reduced). As a result, both chicks in the reduced brood between days 0–15 were full siblings, whereas two chicks in the enlarged brood were unrelated to the other four. However, within brood variation of body size and physiological state did not differ between treatment groups ($p > 0.05$ in all cases; also see Table 3.1), suggesting differences in within brood relatedness between enlarged and reduced broods had little effect on mean brood trait values. Between hatch day and 15 days after hatching, nests were visited every other day to record any chick mortality. At the end of the early developmental phase on day 15, four randomly selected chicks from each enlarged brood, and both chicks from each reduced brood, were weighed (body mass, ± 0.01g), blood sampled, and measured (tarsus, ± 0.01 mm; wing chord, ± 0.5 mm). Following this, two chicks from an enlarged brood were placed in a nearby reduced brood (enlarged-to-reduced). At this time, both chicks originally from the reduced brood were removed and replaced the two chicks that were removed from the enlarged brood (reduced-to-enlarged). Two of the four sampled chicks from the enlarged brood remained in their same nest (no cross-foster). Thus, following cross-fostering on day 15, brood sizes remained the same but two chicks in the enlarged brood had experienced early developmental conditions of a reduced brood, while both chicks in the reduced brood had developed in an enlarged brood. Then, on days 17 and 20 (one day prior to mean fledging age), chicks were subsequently weighed and measured, in addition to blood sampling again on day 20, marking the end of the late developmental phase.

During blood sampling at the end of the early developmental phase (day 15) and late developmental phase (day 20), 100–500 µL of blood (<10% of total blood volume) was taken from the brachial vein using heparinized capillary tubes. On both days, we recorded the time of day and handling times between initial nest disturbance and weighing/blood sampling. However, handling time data was completely confounded by treatment and body mass, as it took longer to process $n = 4$ chicks in each enlarged brood compared with $n = 2$ chicks in each reduced brood. Handling times were consistently low across brood treatments (mean 3.6 ± 0.18 min, < 9 min in all cases) and were equivalent to previous studies in European starlings that showed no relationship between handling times and our measured traits (e.g., Allen *et al.*, 2022); therefore, we did not include handling time when analysing body mass. Both hematocrit and hemoglobin were significantly negatively related to time of day (Pearson's *r*: $p < 0.01$ in both cases). Time of day was therefore controlled for in models analysing hematocrit and hemoglobin. We assayed blood samples for four physiological traits: hematocrit, hemoglobin, dROMs, and OXY. Hematocrit (% packed cell volume) was measured (± 0.01 mm) using digital calipers after whole blood was centrifuged for three minutes at 13,000 *g* (Microspin 24; Vulcon Technologies, Grandview, MO, USA). Hemoglobin (g dl^{-1}) was measured using the cyanomethemoglobin method (Drabkin and Austin, 1932) modified for use with a microplate spectrophotometer (BioTek Powerwave 340; BioTek Instruments, Winooski, VT, USA), measuring 5 µl whole blood diluted in 1.25 ml Drabkin's reagent (D5941; SigmaAldrich Canada, Oakville, Ontario, Canada) at 540 nm absorbance. Reactive oxygen metabolites (mg H_2O_2 dl^{-1}) and total antioxidant titers (µmol HClO ml^{-1}) were measured using dROMs and OXY kits from Diacron International (Grosseto, Tuscany, Italy). All samples were assayed alongside a single pooled plasma sample to calculate an inter-assay coefficient of variation (inter-assay CV: hemoglobin = 1.86%; dROMs = 2.26%; OXY = 5.48%), while an intra-assay CV was calculated from the average CV of replicates within a plate (intra-assay CV: hemoglobin = 1.72%; dROMs = 4.45%; OXY = 2.17%). Red blood cells were sexed by polymerase chain reaction after being sent to HealthGene Laboratory (Concord, Ontario) alongside known adult samples for quality control.

3.3.2. Provisioning observations

At 6–8 days after hatching, video cameras (Canon VIXIA HF R52) were used to obtain provisioning data as nest visit rate. For each nest, two rounds of 30 min provisioning observations were performed on separate days between 9:00 and 14:00 hours. These short observations provide robust measures of individual variation in provisioning effort, as nest boxes show high repeatability of nest visit rate within years (Fowler and Williams, 2015). Provisioning observations between days 6–8 therefore also reflect nest visit rate later in the nestling period, as females show little increase in provisioning between days 5–14 ($r^2 = 0.06$), whereas males show no increase in provisioning over the same period (Enns and Williams, 2022). Videos were analyzed using VLC media player to confirm food delivery and the sex of the provisioning parent via color bands. Provisioning data was calculated by: (i) averaging the number of feeding visits from both days of observations to give a mean overall provisioning rate per nest (mean no. visits / 30 mins), and (ii) dividing the mean number of visits by brood size to give provisioning rate per chick (mean no. visits per chick / 30 mins).

3.3.3. Statistical analysis

All analyses were performed in RStudio v4.2.0 (RStudio, *Inc.*, Boston, MA, USA). We assessed normality of our data using: (i) Shapiro-Wilks tests on all raw trait values; dROMs was not normally distributed and was therefore logarithmically transformed, and (ii) quantile-quantile plots of studentized residuals for our linear mixed-effects models (LMM), removing points of undue influence on a per-model basis which significantly deviated from the normal distribution (fell outside 95% confidence bands). We tested for differences in metrics of provisioning rate between enlarged and reduced broods using two-tailed unpaired *t*-tests. Though we standardized clutch size during incubation, all subsequent models included the original clutch size of the female parent, in addition to nest ID, as random factors to control for variation in parental quality and repeated measures. Partial brood loss (see “Results”) was included as a covariate in all models to control for effects of sibling mortality on development. Sex was included as a covariate where preliminary analyses indicated trait values varied by sex; we controlled for sex in models analysing body mass, tarsus, and wing length.

To compare differences in early development (days 0–15) between manipulated brood sizes, we used LMMs with day 15 trait values and brood treatment (enlarged, reduced) as main effects (e.g., Mass15 ~ Broodsize treatment), calculating marginal means and using paired contrasts (emmeans). For analysis of late developmental trajectories (day 15–20) following cross-fostering, we used LMMs with trait values and age as main effects with treatment (reduced-to-enlarged, enlarged-to-reduced, no cross-foster) as an interaction term (e.g., Mass ~ Age * Cross-fostering treatment). Here we included both the original nest ID and the new nest ID of cross-fostered chick as random factors. Chick ID was also included as a random factor to account for repeated measures. If the age*treatment interaction was significant ($p < 0.05$), we used paired contrasts to compare *changes* in traits during late development (calculated from marginal means as the difference between day 15, 17 and 20 trait values) between cross-fostering treatment groups over each age range (morphological traits: 15–17 and 17–20; physiological traits: 15–20 only). Using the same models but adjusted paired contrasts, we also tested for differences of day 20 trait values between cross-fostering treatment groups at the end of the late developmental phase. Since chick tarsi were fully developed by day 15, showing no significant relationship with age to day 20 (LMM: p = 0.17), we only tested for brood manipulation effects on day 15 tarsus length, while analyses pertaining to cross-fostering did not include chick tarsi data. In-text values are presented as means ± s.e.

3.4. Results

Of the 20 manipulated broods, four enlarged broods demonstrated partial brood reduction. Three broods lost only one chick and are included in subsequent results where partial brood reduction was included as a covariate in all models. However, one enlarged brood lost two chicks, resulting in a brood size of four, which was excluded from subsequent results. In addition, four chicks fledged before day 20 measurements and blood sampling, two that were swapped to a reduced brood and two swapped to an enlarged brood; these chicks are excluded from results pertaining to the late developmental phase. Brood size manipulation results at the end of the early developmental phase (day 15) therefore pertain to n = 10 reduced broods of n = 20 chicks and n = 9 enlarged broods of n = 36 chicks (only four chicks per enlarged brood were measured and blood sampled). Cross-fostering results during the late developmental phase (days 15–20) pertain to n = 18 chicks that remained in an enlarged brood, n = 18 chicks that were swapped to an enlarged brood from a reduced brood, and n = 16 chicks that were swapped to a reduced brood from an enlarged brood.

3.4.1. Provisioning rate

Overall provisioning rate was significantly higher for nests with enlarged broods than reduced broods ($p < 0.01$, $t_{1,12} = 4.36$). Enlarged broods received 7.33 ± 0.73 visits per nest / 30 mins from both parents compared to 3.8 ± 0.35 visits per nest / 30 mins for reduced broods. However, provisioning rate per chick was significantly higher in reduced than enlarged broods ($p = 0.02$, $t_{1,17} = -2.48$), with chicks in reduced broods receiving 1.90 ± 0.18 visits per chick / 30 mins on average compared to 1.32 ± 0.16 visits per chick / 30 mins in enlarged broods.

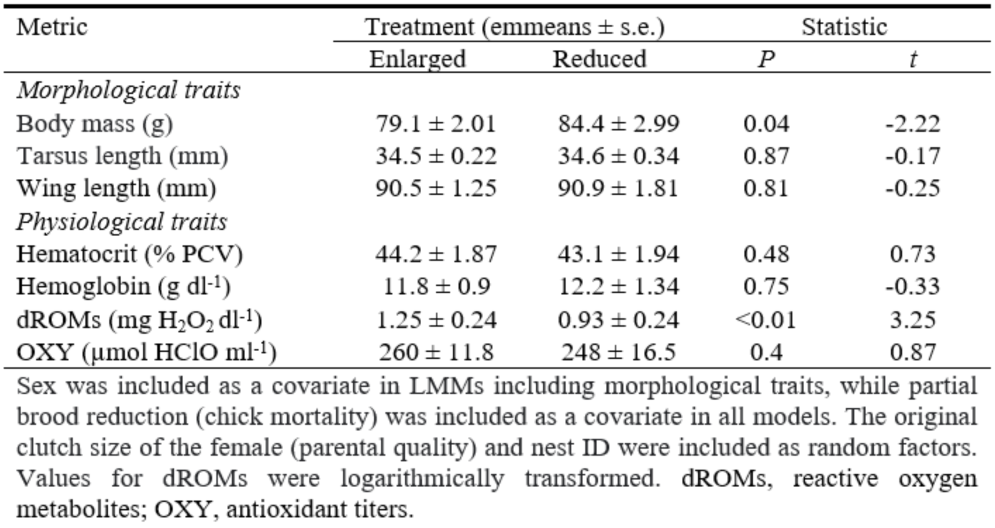
3.4.2. Effects of brood size manipulation on body size and physiological state after early development

Chicks in enlarged broods had significantly lower body mass and higher dROMs at asymptotic mass (day 15) when compared to chicks in reduced broods (Table 3.1). However, neither wing or tarsus (i.e., structural size), hematocrit or hemoglobin (aerobic capacity), nor OXY varied by experimental brood size.

3.4.3. Effects of cross-fostering on body size and physiological state during and after late development

Body mass was significantly negatively related to age during late development (days 15–20; Figure 3.1A; $p < 0.01$, $F_{1,90} = 123$), confirming pre-fledging mass recession, and there was a significant treatment by age interaction ($p = 0.03$, $F_{1,26} = 3.13$). Mass significantly decreased between days 15–17 in chicks cross-fostered from reduced-to-enlarged broods and chicks that were not cross-fostered ($p < 0.01$ in both cases), but with the former losing significantly more mass (8.45 ± 1.47 g) than the latter (3.05 ± 0.75 g; $p < 0.01$, $t_{1,22} = -3.27$). In contrast, there was no significant change in mass over the same period in chicks moved from enlarged-to-reduced broods ($p = 0.61$, $t_{1,14} = -0.52$), differing both from chicks that were not cross-fostered ($p = 0.03$, $t_{1,22} = 2.3$) and chicks that were cross-fostered from reduced-to-enlarged broods ($p < 0.01$, $t_{1,11} = -3.35$).

Table 3.1 Estimated marginal means (emmeans) and paired contrast results of morphological and physiological trait variation by brood size manipulation in European starlings (*Sturnus vulgaris*).



Metric	Treatment (emmeans ± s.e.)		Statistic	
	Enlarged	Reduced	P	t
Morphological traits				
Body mass (g)	79.1 ± 2.01	84.4 ± 2.99	0.04	-2.22
Tarsus length (mm)	34.5 ± 0.22	34.6 ± 0.34	0.87	-0.17
Wing length (mm)	90.5 ± 1.25	90.9 ± 1.81	0.81	-0.25
Physiological traits				
Hematocrit (% PCV)	44.2 ± 1.87	43.1 ± 1.94	0.48	0.73
Hemoglobin (g dl^{-1})	11.8 ± 0.9	12.2 ± 1.34	0.75	-0.33
dROMs (mg H_2O_2 dl^{-1})	1.25 ± 0.24	0.93 ± 0.24	<0.01	3.25
OXY (μmol HClO ml^{-1})	260 ± 11.8	248 ± 16.5	0.4	0.87

Sex was included as a covariate in LMMs including morphological traits, while partial brood reduction (chick mortality) was included as a covariate in all models. The original clutch size of the female (parental quality) and nest ID were included as random factors. Values for dROMs were logarithmically transformed. dROMs, reactive oxygen metabolites; OXY, antioxidant titers.

In contrast, changes in mass between days 17–20 were similar for all treatments ($p > 0.28$ in all cases). As a result, pre-fledging (day 20) mass at the end of the late developmental phase was significantly greater in chicks moved from enlarged-to-reduced broods (76.5 ± 2.02 g) compared to chicks that were not cross-fostered (72 ± 1.74 g; $p = 0.04$, $t_{1,40} = -2.15$). Pre-fledging mass in chicks cross-fostered from reduced-to-enlarged broods (73.1 ± 2.13 g) was intermediate between that of chicks in the other treatment (pair-wise contrast, $p > 0.07$ in both cases). Wing length was significantly positively related to age ($p < 0.01$, $F_{1,90} = 6040$), growing 19 ± 0.17 mm on average between days 15–20 (Figure 3.1B), but did not vary by treatment ($p = 0.73$, $F_{1,37} = 0.31$) nor was there a treatment*age interaction ($p = 0.15$, $F_{1,21} = 1.88$).

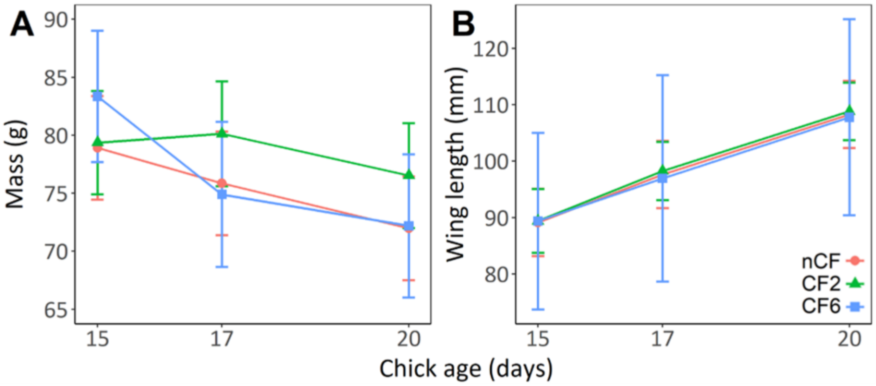


Figure 3.1 **Morphological traits approaching fledging by cross-fostering treatment in European starling (*Sturnus vulgaris*) nestlings. Chick age (A, B) in relation to mass (A) and wing length (B). Shape and color correspond to cross-fostering treatment on day 15; chicks remained in their cross-fostered broods until fledge (day 21). Changes in mass, but not wing length, varied by cross-fostering treatment and by age (a significant interaction). CF6 and nCF treatments demonstrated significant mass recession between days 15–17, whereas the CF2 treatment showed no change in mass over the same period. However, all treatments demonstrated significant mass recession between days 17–20 and the amount of mass lost did not differ by treatment. Consequently, CF2 chicks fledged heavier than the other two treatments and there was no difference in body mass between CF6 and nCF treatments at fledging (see "Results"). CF6, swapped from a reduced brood to an enlarged brood; CF2, swapped from an enlarged brood to a reduced brood; nCF, remained in an enlarged brood.**

Hematocrit and hemoglobin were both significantly positively related to age (Figure 3.2A,B; $p < 0.01$ in both cases) and changes in these traits to day 20 did not differ between treatments ($p > 0.64$ in both cases). Similarly, dROMs was significantly positively related to age (Figure 3.2C; $p < 0.01$, $F_{1,50} = 12.5$) and there was no treatment*age interaction ($p = 0.93$, $F_{1,14} = 0.06$). However, dROMs did vary significantly by treatment overall (an intercept effect; $p = 0.02$, $F_{1,46} = 4.22$), as dROMs of chicks cross-fostered from reduced-to-enlarged broods was lower than both chicks moved from

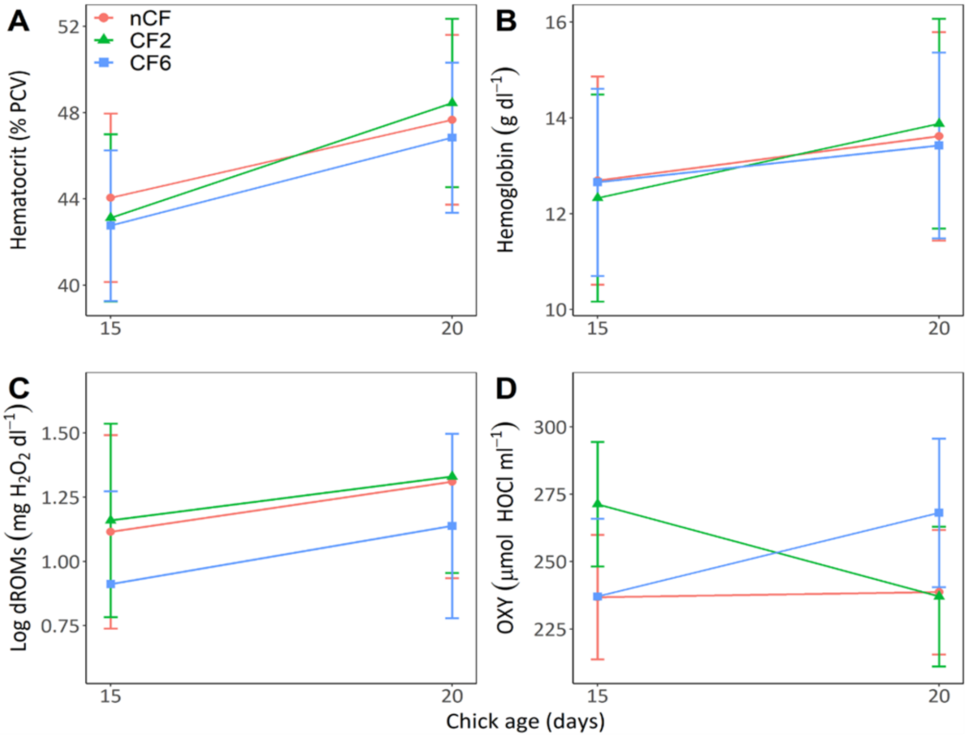


Figure 3.2 **Physiological traits approaching fledging by cross-fostering treatment. Chick age (A-D) in relation to hematocrit (A), hemoglobin (B), log dROMs (C), and OXY (D). Traits were significantly positively related to age in (A-C) but not (D). Changes in OXY between days 15–20 were significantly different between CF2 and CF6 treatments. Neither treatments differed significantly from the nCF treatment. OXY significantly decreased between days 15–20 in the CF2 treatment, whereas OXY significantly increased in the CF6 treatment over the same period. The nCF treatment showed no significant change in OXY between days 15–20. Changes in hematocrit, hemoglobin, and dROMs did not vary significantly between groups (see "Results"). dROMs, reactive oxygen metabolites; OXY, antioxidant titers.**

enlarged-to-reduced broods ($p = 0.02$, $t_{1,19} = 2.63$) and chicks that were not cross-fostered ($p = 0.04$, $t_{1,24} = 2.16$). There was no difference of dROMs between chicks cross-fostered from enlarged-to-reduced broods and chicks that were not cross-fostered ($p = 0.68$, $t_{1,34} = -0.42$).

Overall, OXY showed no significant relationship with age (Figure 3.2D; $p = 0.95$, $F_{1,95} < 0.01$), but there was a significant treatment*age interaction ($p < 0.01$, $F_{1,95} = 5.06$). Changes in OXY between days 15–20 were significantly different between chicks cross-fostered from enlarged-to-reduced broods and chicks moved from reduced-to-enlarged broods ($p < 0.01$, $t_{1,19} = 3.1$), but there was no difference between chicks that were not cross-fostered and chicks cross-fostered from enlarged-to-reduced broods ($p = 0.07$, $t_{1,47} = -1.88$) nor chicks moved from reduced-to-enlarged broods ($p = 0.14$, $t_{1,49} = 1.48$). OXY significantly increased between days 15–20 in chicks cross-fostered from reduced-to-enlarged broods ($p = 0.04$, $t_{1,41} = -2.11$), whereas OXY decreased in chicks cross-fostered from enlarged-to-reduced broods over the same period ($p = 0.02$, $t_{1,36} = 2.44$). There was no change in OXY of chicks that were not cross-fostered ($p = 0.89$, $t_{1,44} = -0.14$). Pre-fledging OXY on day 20 was therefore greater in chicks cross-fostered from reduced-to-enlarged broods compared to chicks moved from enlarged-to-reduced broods ($p < 0.05$, $t_{1,52} = -2.02$) and chicks that were not cross-fostered ($p < 0.05$, $t_{1,66} = -2$). There was no difference of day 20 OXY between chicks that were moved from enlarged-to-reduced broods and chicks that were not cross-fostered ($p = 0.91$, $t_{1,45} = 0.12$).

3.5. Discussion

Here we experimentally tested the robustness of morphological and physiological traits to environmental variation in two natal developmental stages (early development up to asymptotic mass on day 15 versus late development during pre-fledging mass recession between days 15–20), by manipulating brood size at hatch and subsequently cross-fostering chicks at the end of the early developmental phase. Brood size manipulation at hatch had a significant effect on asymptotic mass, with chicks in reduced broods being heavier than chicks in enlarged broods, but brood size manipulation had no effect on other metrics of morphological growth (tarsus, wing length) or putative physiological developmental traits (hematocrit, hemoglobin). However, the smaller (relative to reduced broods) chicks from enlarged broods had higher plasma reactive oxygen metabolite levels than the larger reduced brood chicks at the end of the early developmental phase. Subsequent cross-fostering of chicks between reduced and enlarged broods (i.e., from a ‘good’ to ‘poor’ natal environment and vice versa) at the beginning of the late developmental phase affected the pattern of pre-fledging mass recession. Chicks cross-fostered from enlarged-to-reduced broods did not lose mass between days 15 and 17 (contrary to the normal pattern of pre-fledging mass recession, Allen *et al*., 2022) and ended up heavier at fledging (day 20). In contrast, chicks that originated from reduced broods (heaviest at asymptotic mass) lost the most mass between days 15 and 17 after being cross-fostered to enlarged broods and showed no difference in mass at fledging from chicks that were not cross-fostered. However, cross-fostering at asymptotic mass did not affect subsequent late developmental changes in wing growth, hematocrit, hemoglobin, or reactive oxygen metabolites to day 20. Consequently, chicks that originated from reduced broods (prior to cross-fostering) maintained significantly lower reactive oxygen metabolite levels to fledging. In contrast, antioxidant capacity varied significantly by treatment, as chicks cross-fostered from reduced-to-enlarged broods increased their antioxidant capacity during late development, whereas antioxidant capacity in chicks cross-fostered from enlarged-to-reduced broods decreased over the same period. Chicks cross-fostered from reduced-to-enlarged broods therefore fledged with greater antioxidant capacity than the other two treatments.

Chicks in reduced brood sizes were provisioned more frequently per chick than those in enlarged broods, potentially indicating the former had higher food intake. The combination of increased provisioning and decreased sibling competition implies reduced broods experienced more favourable early developmental conditions. In agreement, reduced brood chicks were heavier at asymptotic mass on day 15, supporting previous work that has shown marked sensitivity of body mass to the natal environment (Saino *et al*., 1997; Nilsson and Gårdmark, 2001; Ilmonen *et al*., 2003; Siefferman and Hill, 2007; Romero-Haro and Alonso-Alvarez, 2020; Arct *et al*., 2022). In contrast, structural traits (tarsus, wing length) demonstrated an insensitivity to variation in experimental brood size during early development, consistent with previous studies showing wing growth that is robust to environmental variation (Nilsson and Gårdmark, 2001; Ilmonen *et al*., 2003; Siefferman and Hill, 2007; Bourgeon *et al*., 2011; Boonekamp *et al*., 2017; Cornell and Williams, 2017). Wing length has been shown to have strong associations with post-fledging survival across species (Martin *et al*., 2018; Jones and Ward, 2020), which likely selects for the general canalization of wing growth observed during early development here. The corresponding absence of variation in tarsus length on day 15 in response to experimental brood size suggests it too is a canalized trait in this species. Indeed, previous studies have shown how tarsus length seemingly varies the least out of many morphological traits in response to brood size manipulations (Burness *et al*., 2000; Bourgeon *et al*., 2011; Romero-Haro and Alonso-Alvarez, 2020). However, the reason for its apparent canalization is unclear, given that it is difficult to conceive how variation in tarsus length could strongly affect fitness since it is likely independent of juvenile flight ability. Instead, perhaps the insensitivity of tarsus and wing length to environmental variation during early development reflects a general canalization of structural growth (e.g., Moe *et al*., 2004), as development of these traits may be maintained and/or promoted via similar mechanisms. Alternatively, tarsus length could be an important component of fitness prior to the acquisition of flight, potentially involved in sibling competition in the nest, which could explain why European starlings attain adult maturity of tarsus length so early in development.

Maturation of hematocrit and hemoglobin during early development was also insensitive to experimental brood size, adding to the complex relationship between aerobic capacity and developmental context apparent from previous studies (Potti *et al*., 1999; Burness *et al.*, 2000; Simon *et al*., 2005; Ardia, 2013; Sakaluk *et al*., 2014; Cornell and Williams, 2017). Previous work seems to generally suggest a greater sensitivity of aerobic capacity to environmental variation when compared to morphological traits (Potti *et al*., 1999; Ardia, 2013; Cornell and Williams, 2017), although we found no evidence for that here. Instead, we observed a robustness of aerobic capacity to environmental variation which was comparable to that of structural growth, potentially suggestive of similar links to fitness and canalization of some physiological traits as shown previously (Boonekamp *et al*., 2018). Indeed, hematocrit and hemoglobin have been shown to significantly influence flight performance in European starling fledglings (Cornell *et al*., 2017), a significant determinant of juvenile survival (Jones and Ward, 2020), likely promoting the canalization of aerobic capacity during early development observed in this study. Differences in the sensitivity of aerobic capacity to environmental variation observed in previous studies might then reflect complexity in the relationship between aerobic capacity and survival (e.g., Bowers *et al*., 2014; Brown *et al*., 2021) and variation in sampling age (especially if aerobic capacity is measured once developmental maturity has been achieved). Here, due to the immaturity of hematocrit and hemoglobin at fledging in European starlings relative to adult values (Cornell *et al*., 2017), we interpret higher values of aerobic capacity as indicative of more rapid physiological development (or representative of individual quality; Minias, 2015) and potentially increased fitness (Thomas *et al*., 2007).

The observed increase in reactive oxygen metabolites in enlarged brood chicks suggests their oxidative status decreased during early development (Monaghan *et al*., 2009; Murphy *et al*., 2011), especially considering antioxidant capacity did not reflect variation in reactive oxygen metabolites at day 15, as would be expected if chicks were attempting to reduce oxidative stress (Monaghan *et al*., 2009). Instead, antioxidant capacity was consistent between experimental brood sizes, as shown previously (Gil *et al*., 2019). Furthermore, we found no evidence that oxidative status was linked to increased developmental rates (Metcalfe and Alonso-Alvarez, 2010), as structural size and aerobic capacity were similar between treatments after the early developmental phase, while the heavier reduced broods paradoxically had lower reactive oxygen metabolites. Instead, Cornell and Williams (2017) proposed that maintaining ‘hardwired’ growth trajectories in low-quality environments might incur oxidative costs, and this is supported here, as we observed increased reactive oxygen metabolite production in less favourable developmental conditions (enlarged broods) but no change in structural or physiological maturation between experimental brood sizes. Thus, our data suggest that juvenile birds trade-off canalization of structural and physiological development during early growth phases against oxidative status in poor developmental contexts (e.g., increased natal brood size).

Consistent with early development up to asymptotic mass, body mass after cross-fostering (during pre-fledging mass recession i.e., the late developmental phase) showed marked environmental sensitivity, while the insensitivity of wing growth and physiological maturation (aerobic capacity) to environmental conditions was maintained. Chicks cross-fostered to more favourable conditions (reduced brood) seemingly delayed the onset of mass recession to day 17 and lost the least mass in the approach to fledging, while chicks cross-fostered to enlarged broods displayed greater mass recession which began two days earlier. The comparative flexibility of body mass and mass recession (cf. structural traits) is likely advantageous during development and may explain the consistent sensitivity of body mass to environmental variation across different developmental phases. For example, greater body mass may help buffer against variable conditions earlier in the nestling period (Lack, 1968; Ricklefs, 1990; Phillips and Hamer, 1999; De Zwaan *et al*., 2020), but declines in body mass are promoted prior to fledging to optimize wing-loading and improve flight performance at fledging (Martins, 1997; Wright *et al*., 2007; Goodpaster and Ritchison, 2014). Consequently, despite chicks originating from reduced broods being heavier at asymptotic mass (prior to cross-fostering), their greater body mass was not maintained during pre-fledging mass recession after being cross-fostered to less favourable conditions (enlarged brood), while chicks that were cross-fostered from enlarged-to-reduced broods fledged heavier (despite being lighter at asymptotic mass five days prior), potentially promoting post-fledging survival (Magrath, 1991; Schwagmeyer and Mock, 2008; Ronget *et al*., 2018). Our data therefore suggest the positive effects of favourable early developmental conditions on body mass do not carry over between growth phases when environmental context changes.

In contrast, the effects of experimental brood size on reactive oxygen metabolites during early development appeared to carry over into the late developmental period of mass recession, as there were no changes in reactive oxygen metabolites associated with cross-fostering between low- and high-quality environments. Instead, reactive oxygen metabolites remained lowest in chicks that had originated in reduced broods and were cross-fostered to enlarged broods (an intercept effect), while chicks that originated from enlarged broods showed no improvement in their oxidative state when cross-fostered to reduced broods. Thus, chicks that experienced a poor environmental context during early development continued to show subsequent markers of oxidative stress up to fledging, despite being cross-fostered to more favourable developmental conditions on day 15. Since pre-fledging oxidative stress has been shown to decrease recruitment in European shags (Noguera *et al*., 2012), the observed carry over of increased reactive oxygen metabolite levels across growth phases may suggest negative fitness consequences of poor environmental conditions during early development, even if conditions improve later in development. Like at the end of the early developmental phase, antioxidant capacity during late development did not reflect variation in reactive oxygen metabolites. However, in contrast to early development, antioxidant capacity in the approach to fledging demonstrated a variable relationship with environmental conditions. Antioxidant capacity in chicks transferred into reduced broods decreased to day 20, while chicks cross-fostered to enlarged broods subsequently increased their antioxidant capacity. Thus, antioxidant capacity trajectories during late development specifically reflected a *change* in developmental context relative to the natal environment, as antioxidant capacity did not vary by age in chicks that were not cross-fostered. This could suggest chicks made investment decisions relating to antioxidant capacity that depended upon the 'new' environmental conditions experienced during late development. These data therefore suggest reactive oxygen metabolites and antioxidant capacity possess varied relationships with environmental context and developmental stage: poor conditions early in development may have lasting effects on oxidative state that carry over between developmental stages, while antioxidant capacity is seemingly more likely to reflect the ontogenetic environment later in development. Collectively, this work shows the potential insensitivity of several developmental traits (structural growth, aerobic capacity) to environmental variation, as well as the potential oxidative costs associated with canalization in specific developmental contexts, while highlighting the importance of developmental stage when interpreting environmental effects and changes in oxidative status.

3.6. Acknowledgements

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Chapter 4.

Pre-fledging ‘exercise’ in a passerine bird

4.1. Chapter abstract

Anecdotal observations of flight preparation behaviours (e.g., wing flapping) are common in many larger birds (e.g., seabirds, raptors) with open nests, but very little is known about pre-fledging exercise in small, cavity-nesting passerine birds. Any putative exercise has rarely been quantified, making their adaptive function and role in other developmental processes associated with flight (e.g., pre-fledging mass recession) uncertain. Here, we used video cameras placed in nestboxes of European starlings (*Sturnus vulgaris*) to characterise and quantify (duration and number of bouts) exercise behaviours of nestlings approaching fledging. In addition, we obtained nest provisioning rates and repeated measures of chick size to test for correlations between any observed exercise with variation in developmental trajectories and food availability. Chicks demonstrated two exercise behaviours: wing extensions and wing flapping, and these had contrasting relationships with chick age, morphology (wing growth and length), and provisioning rate. Wing extension bouts were longer and more frequent at younger ages and showed no change in the approach to fledging, while wing flapping was initially rare but increased significantly as chicks aged. Neither exercise was related to mass recession as would be expected if increased activity was a mechanism for pre-fledging mass loss. However, both the mean daily duration and number of wing extension bouts were positively correlated with wing length at fledging, while the mean daily number of wing flapping bouts was negatively correlated with wing growth. Furthermore, wing flapping (both mean daily duration and number of bouts) was positively correlated with provisioning rate, while wing extensions were unrelated to provisioning rate. Our data provide novel evidence for pre-fledging exercise in a passerine bird and could suggest independent functions (e.g., growth versus motor skill acquisition) of wing extension and wing flapping exercises, highlighting the potential importance of juvenile behaviour during this understudied life-stage.

4.2. Introduction

Increased activity levels in humans are positively correlated with physical fitness (Hoppeler *et al*., 1985; Pollok *et al*., 1987; Malina, 1996). Through exercise, humans may improve their performance and reduce the risk of injury when met with an increased workload (e.g., in sport; Armstrong, 1984; Appell *et al*., 1992). These benefits may be promoted by exercise through motor skill acquisition and/or the enhancement of morphological and physiological traits. Similarly, laboratory studies have shown how animals may benefit from increased activity (reviewed in Yap *et al*., 2017), exhibiting increased aerobic capacity and fuel metabolism in response to exercise in wind tunnels (birds), swim tunnels (fish) and running wheels (rodents). However, markers of oxidative stress and decreased immune function suggest costs of experimentally induced exercise in animals that might predict that exercise would be uncommon in more ecologically relevant settings (Yap *et al*., 2017). In agreement, empirical evidence of exercise in free-living animals is rare, although exercise-like behaviours appear more common prior to/during the transition from parental-dependence to independence in juveniles. Here, immature mammals will often engage in 'play' (Muller-Schwarze *et al*., 1982; Sharpe *et al*., 2002; Nunes *et al*., 2004; Cameron *et al*., 2008), analogous to exercise in many cases, while nesting birds demonstrate putative flight-preparation exercises (e.g., wing flapping; Warham, 1990; Wright *et al*., 2006; Yoda *et al*., 2017). However, these exercise behaviours have seldom been quantified in birds, leaving their adaptive function uncertain.

While 'play' in mammals involves interaction with the environment and sociality which might better promote motor skill acquisition (e.g., Berghänel *et al*., 2015), putative exercise behaviours in birds are performed independently and may be confined to the nest (but see Yoda *et al*., 2017). Thus, it might be predicted that the function of juvenile exercise in birds is to promote the development of traits related to flight ability. For example, many species demonstrate mass-overshoot recession growth profiles (Phillips and Hamer, 1999; Gray and Hamer, 2001; Masello and Quillfeldt, 2002), where nestlings achieve (and often exceed) adult mass relatively early in development, only to lose a significant portion of that mass in the approach to fledging as chicks putatively optimize their wing-loading in preparation for flight (Wright *et al*., 2006; Goodpaster and Ritchison, 2014). The observation of wing flapping and 'push-up' exercises in swifts approaching fledging was suggested to play a role in the observed facultative adjustment of mass recession (Wright *et al*., 2006), where increasing activity levels might be partly utilized to facilitate weight-loss, as in humans. This idea has yet to be empirically tested and the mechanism by which chicks supposedly control mass recession remains unknown. Alternatively, pre-fledging exercise could promote the growth of bones and muscles that support flight (Carrier and Leon, 1990), as suggested in burrow-nesting streaked shearwaters (*Calonectris leucomelas*, Yoda *et al*., 2017), yet the extent to which small, cavity-nesting passerine birds engage in pre-fledging exercise (cf. seabirds, raptors) is unclear.

In this study, we observed and analysed pre-fledging nestling behaviour in a cavity-nesting passerine, the European starling (*Sturnus vulgaris*). We took repeated measures of chick body size (mass, tarsus, wing length) to test if exercise is not only correlated with absolute size (as in Yoda *et al*., 2017), but if it is directly related to changes in morphological traits (i.e., mass recession, wing growth). Furthermore, since juvenile exercise may be facilitated by excess energy (e.g., “surplus resource hypothesis” for locomotor play; Graham and Burghardt, 2010), we obtained nest provisioning rates (number of visits by parents with food / 30 mins) to test if exercise is correlated with parental food supply, independent of associations between provisioning rate and nestling size. We predicted a negative relationship between pre-fledging exercise and changes in mass (i.e., more exercise would increase mass loss), based on the hypothesis that increased activity levels prior to fledging are a mechanism for pre-fledging mass recession. In contrast, we predicted that the duration and number of bouts of exercise behaviours would be positively related to wing growth, as exercise would promote muscle and bone development (Carrier and Leon, 1990). Finally, we predicted that nests which experience higher provisioning rates would exercise longer and more frequently, as surplus energy of chicks in these nests would facilitate adaptive increases of activity levels (Graham and Burghardt, 2010).

4.3. Methods

Field work was conducted over two breeding seasons (2021–2022) on a nest-box breeding population of European starlings at Davistead Farm, Langley, British Columbia, Canada (49°10'N, 122°50'W). Egg-laying is checked daily at 150 nestboxes between March and April to determine lay date and clutch size, after which nests are monitored until fledging or failure. Between days 6–8 after hatching, we obtained provisioning data using video cameras (Canon VIXIA HF R52) to measure food delivery rate over two 30-minute observations. On separate days, between 9:00 and 14:00 hours, cameras were placed at 15 m from the focal nest in open fields or hidden 5 m from a nest within farmyard buildings. VLC media player was used to analyze provisioning observation videos to identify the sex of the provisioning parent and confirm food delivery, although we did not quantify the amount of food delivered. Nestboxes show high repeatability of nest visit rate within years (Fowler and Williams, 2015) and show little change in provisioning by chick age (Enns and Williams, 2022; Allen, unpublished data). Thus, provisioning rates between days 6–8 provide robust measures of individual variation in provisioning effort which are consistent through the nestling period. We calculated provisioning rates as the mean number of visits by both parents divided by brood size (mean no. visits per chick / 30 mins).

To observe chick activity approaching fledging, we fixed video cameras (M80 Air 4K Action Camera) to the inside of nestbox lids daily between days 16–20 (greater than 90% of chicks fledge on day 21; Cornell *et al*., 2017), recording chick behaviour from directly above the nest. Fully charged cameras were placed in nestboxes between 9:00 and 15:00 hours and were left until the battery died (mean observation duration = 62.3 ± 2.15 mins). Chicks were weighed (body mass, ± 0.01g) and measured (tarsus, ± 0.01 mm; wing chord, ± 0.5 mm) on days 15 (asymptotic mass), prior to all observations. At this time, all chicks were colour-banded and non-toxic metallic ink was used to mark the back of the neck of each chick, allowing individual identification of chicks from video recordings. On days 17 and 20, as cameras were retrieved after video observations, chicks were weighed and measured again. On each day, we recorded handling times between initial nest disturbance and weighing (mean handling time = 4.15 ± 0.27 min) and time of day. Neither handling time nor time of day was related to body mass ($p > 0.05$ in all cases).

Following preliminary analysis of observations from 2020 (Allen, unpublished data), we identified two putative ‘exercise’ behaviours approaching fledging: wing flapping and wing extensions. Wing extensions involved a chick extending one or both wings out more than 50% of its total wing length without subsequently flapping (Figure 4.1). We used VLC media player to quantify both behaviours; for each day we recorded the total duration of activity and number of bouts of each chick. From these data, for every chick we calculated two activity metrics for each exercise behaviour: (i) mean activity duration (daily duration / 60 mins averaged across days 16–20), and (ii) mean number of bouts (daily count / 60 mins averaged across days 16–20). Of the *n* = 55 video observations performed, one was excluded due to adult males fighting in the nest, while another one was excluded as the nest had fledged before day 20 (final day of observation). Additionally, five observations were excluded due to poor lighting (weather, chicks blocking nestbox hole) that made chick identification uncertain, leaving *n* = 48 observations of *n* = 46 chicks in *n* = 11 nests for final analysis.

4.3.1. Statistical analysis

All analyses were performed in RStudio v4.2.0 (RStudio, *Inc.*, Boston, MA, USA). We assessed normality of our data using Shapiro-Wilks test on all raw trait values and activity metrics; measures of wing extensions (duration and bouts) were not normally distributed and were logarithmically transformed. In addition, measures of chick wing length, as well as wing flapping (duration and bouts), were not normally distributed but showed no improvement when transformed. We therefore used quantile-quantile plots of studentized residuals for our linear mixed-effects models (LMM) to assess normality and remove points of undue influence (fell outside 95% confidence bands) on a per-model basis; a maximum of six chicks were removed from any single model. Chick development between days 15–20 was analysed using LMMs with trait (mass, wing length) and age as main effects, in addition to chick ID nested within nest ID and year as random factors. To test for changes in activity between days 16–20, we used LMMs with the activity metric (daily duration or bout / 60 mins) and age as main effects and included chick ID nested within nest ID and year as random factors. Brood size was unrelated to body mass at fledging ($p = 0.93$) but was significantly related to wing length on day 20 ($p = 0.04$) and was therefore included as a covariate in all models pertaining to day 20 wing length. Since chick tarsi are fully developed by day 15 in European starlings (Allen *et al.*, 2022), we included tarsus length in all models to control for chick size.

We tested whether measures of wing flapping and extensions for individual chicks (mean daily bout duration and mean daily number of bouts) were correlated with pre-fledging developmental trajectories (mass recession, wing growth) and size at fledging (body mass, wing length), using LMMs that included activity metrics and the *change* in trait values between days 15–20 or trait values at fledging (day 20) as main effects. We also tested for correlations between provisioning rate and chick activity using LMMs with provisioning rate and metrics of wing flapping and extensions as main effects, while including chick body mass and tarsus length as covariates to control for relationships between provisioning and chick body size. The total number of observations, but not the duration of those observations, was included as a covariate in models analysing the mean daily bout duration or number of bouts, since these metrics were calculated per unit time (/ 60 mins). Nest ID and year were included in all models as random factors. In-text values are presented as means ± s.e.

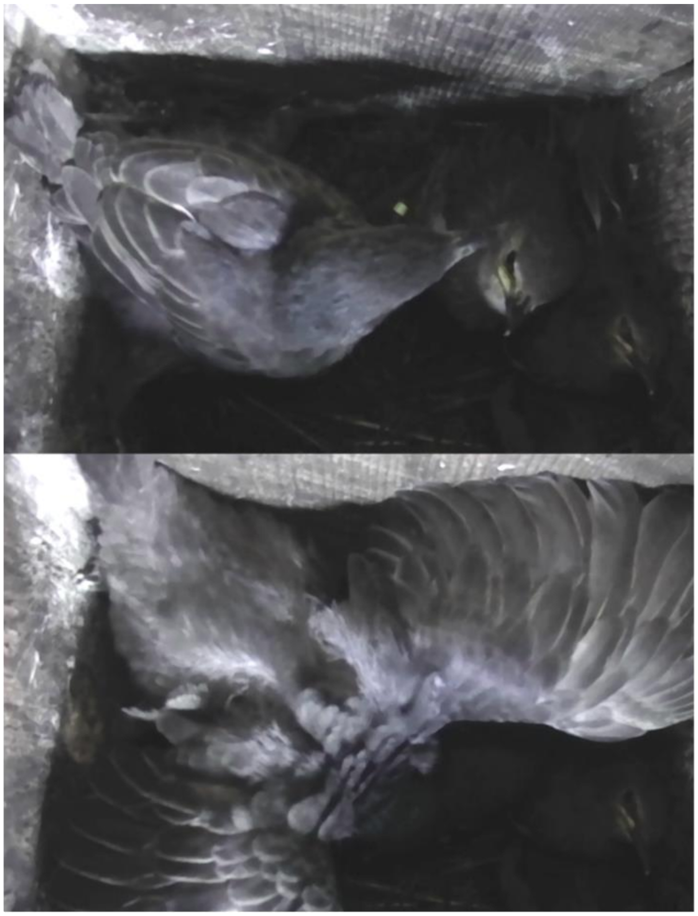

Figure 4.1 **An 18-day old European starling (*Sturnus vulgaris*) demonstrates wing extensions, three days before fledging. Chicks hold their wings in an extended position without flapping, with bouts lasting up to 20 seconds.**

4.4. Results

Mass was significantly negatively related to age ($p < 0.01$, $F_{1,80} = 20.7$), as chicks displayed mass recession approaching fledging, losing -2.91 ± 0.83 g on average between days 15–20. Conversely, wing length was significantly positively related to age ($p < 0.01$, $F_{1,79} = 3804$), increasing by 20.3 ± 0.32 mm on average between days 15–20. Daily wing flapping duration between days 16–20 averaged at 5.49 ± 0.47 seconds / 60

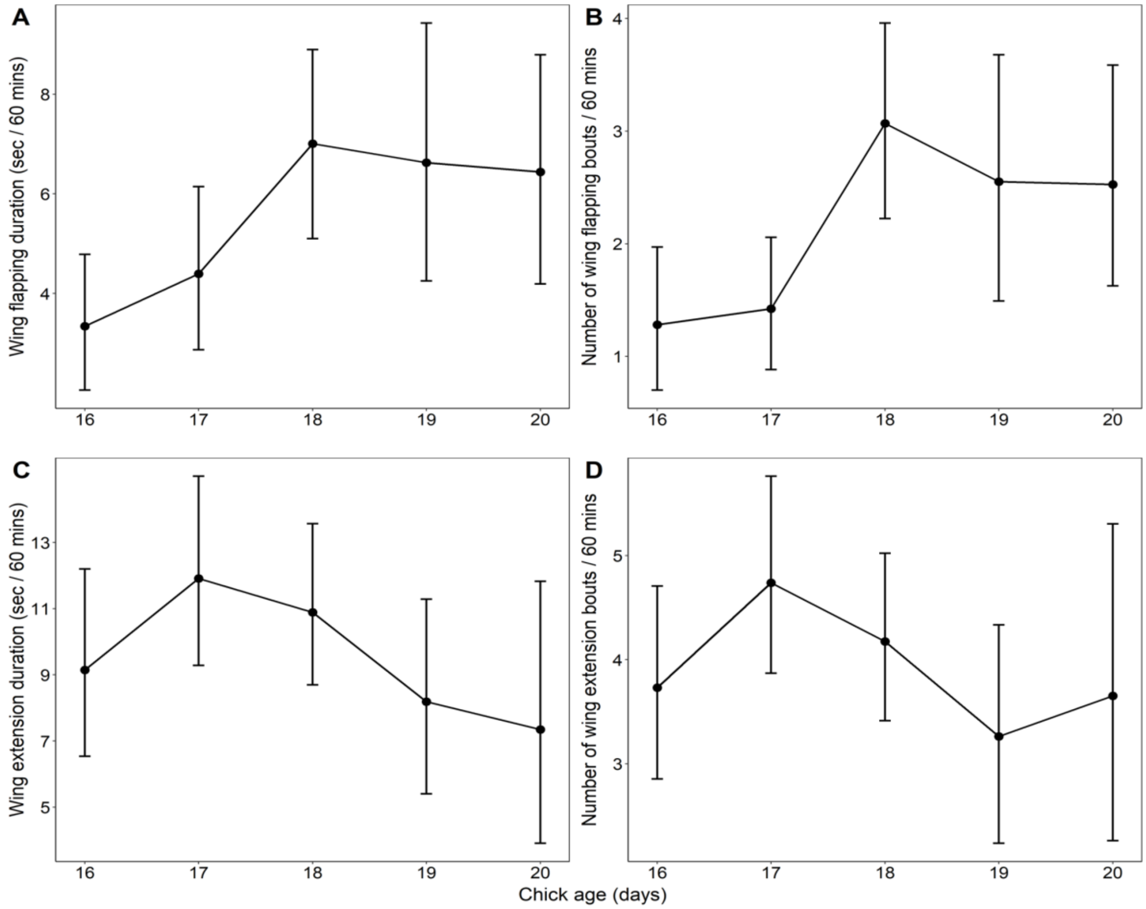

Figure 4.2 **Activity approaching fledging in nestling European starlings (*Sturnus vulgaris*). Chick age (A-D) in relation to wing flapping duration (A), number of wing flapping bouts (B), wing extension duration (C), and number of wing extension bouts (D). Both metrics of wing flapping (A, B) increased significantly in the approach to fledging, whereas wing extensions (C, D) were unrelated to chick age (see "Results").**

mins over a mean number of 2.14 ± 0.2 bouts / 60 mins per day. In addition, daily wing extension duration between days 16–20 averaged at 11.1 ± 1.1 seconds / 60 mins over a mean number of 4.25 ± 0.33 bouts / 60 mins per day. Neither activity, both in terms of duration and number of bouts, varied by time of day ($p > 0.09$ in all cases), although 43 of the $n = 48$ total behavioural observations were performed between 9:00 and 11:00. In addition, neither activity varied by brood size ($p > 0.09$ in all cases). There was a significant increase in wing flapping approaching fledging (Figure 4.2A,B), both in terms of daily duration ($p = 0.03$, $F_{1,149} = 2.84$) and number of bouts ($p < 0.01$, $F_{1,143} = 5.33$). The average time chicks spent wing flapping on day 16 was 3.34 ± 0.73 seconds / 60 mins, increasing to 6.44 ± 1.22 seconds / 60 mins on day 20. Similarly, the average number of wing flapping bouts increased from 1.28 ± 0.32 / 60 mins on day 16 to 2.53 ± 0.51 / 60 mins on day 20. In contrast, wing extensions showed no significant relationship with age (Figure 4.2C,D), neither in terms of duration ($p = 0.37$, $F_{1,151} = 1.08$) nor number of bouts ($p = 0.16$, $F_{1,153} = 1.66$).

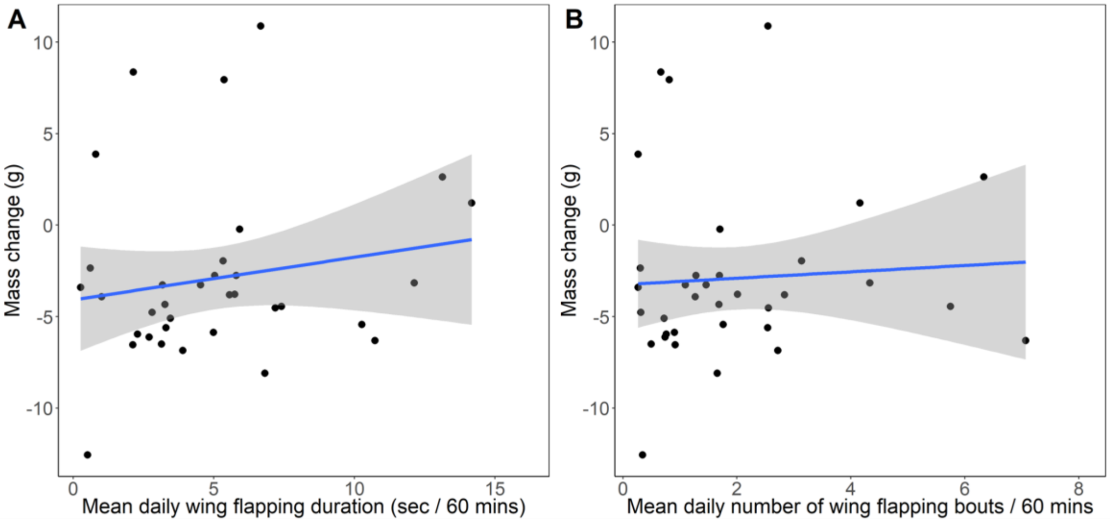

Figure 4.3 **Wing flapping approaching fledging in relation to pre-fledging mass recession. Change in mass between days 15–20 (A, B) versus mean daily flapping duration (A) and mean daily number of wing flapping bouts (B). Each point represents a single chick and the average calculated from daily measures of wing flapping activity between days 16–20. Neither relationship (A, B) was significant (see "Results").**

Change in mass between days 15–20 was unrelated to both metrics of wing extensions ($p > 0.66$ in both cases) and wing flapping (Figure 4.3; $p > 0.17$ in all cases). However, wing growth was negatively related to the mean number of wing flapping bouts between days 16–20 ($p < 0.01$, $F_{1,22} = 10.5$), but not the mean daily duration of wing flapping ($p = 0.62$, $F_{1,30} = 0.25$). In contrast, both the mean duration and number of bouts of wing extensions between days 16–20 were positively associated with wing length at fledging (Figure 4.4; $p < 0.03$ in both cases). However, wing length at fledging was unrelated to wing flapping ($p > 0.64$ in both cases), while neither exercise was correlated with body mass at fledging ($p > 0.26$ in all cases). Provisioning rate was significantly positively correlated with both mean wing flapping duration ($p = 0.02$, $F_{1,11} = 6.73$) and mean number of wing flapping bouts ($p < 0.01$, $F_{1,11} = 9.81$), independent of relationships between provisioning rate and body size. However, provisioning rate was not related to either measure of wing extensions ($p > 0.06$ in all cases).

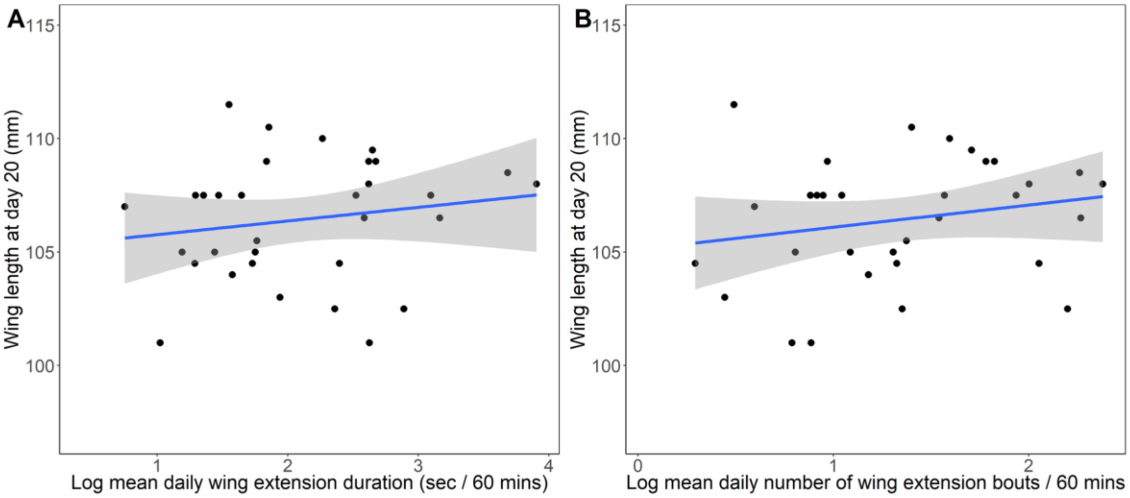

Figure 4.4 **Wing extensions approaching fledging in relation to wing length at fledging. Wing length at day 20 (A, B) versus log mean daily wing extension duration (A) and log mean daily number of wing extension bouts (B). Each point represents a single chick and the average calculated from daily measures of wing extension activity between days 16–20. Both relationships (A, B) were significant (see "Results").**

4.5. Discussion

We investigated nestling behaviour in European starlings and present, to the best of our knowledge, the first ever quantification of pre-fledging exercise in a cavity-nesting passerine bird. We identified two potential ‘exercises’: wing extensions and wing flapping, which demonstrated contrasting relationships with chick age and morphology (wing growth and length), suggestive of independent adaptive functions of both exercises (promoting growth and motor skill acquisition, respectively). This was supported by variation between exercises in their relationship with provisioning rate, suggesting wing flapping exercises, but not wing extensions, may be conditional upon surplus energy reserves. The correlative nature of these data do however limit our interpretations, as it is difficult to determine whether, for example, increased exercise promotes growth or if increased growth promotes more exercise. Indeed, despite finding systematic variation in both putative exercise behaviours, these occurred at very low frequencies and for short durations, perhaps making “exercise-induced” changes in phenotype unlikely. Furthermore, although we were cautious of quantifying any ‘exercise’ that might be better explained by balancing and movement around the nest, the positive correlation between provisioning rate and wing flapping could be an artifact of increased activity associated with more frequent visits to the nest by parents. Nonetheless, we broadly show that passerine birds, like other open-nesting birds, might perform pre-fledging exercises and that in European starlings, this might have consequences for juvenile phenotype.

Wing extensions were performed for longer and more frequently than wing flapping exercises between days 16–20, although activity levels were generally low with < 1% of time dedicated to exercise on average per 60 minutes. The difference in mean duration and frequency of each exercise across the pre-fledging period was driven by relatively low levels of wing flapping (cf. wing extensions) when chicks were younger. In contrast, the mean daily duration and number of bouts of wing extensions were relatively high from a young age and showed no change across the pre-fledging period. The relative importance of wing extensions for younger chicks could suggest this exercise has a function relating to growth and physiological maturation, as it was observed at high rates (relative to wing flapping) long before the need for locomotor skills, while wing flapping only increased as chicks approached their first flight. In agreement, both measures of wing extensions were positively correlated with wing length at fledging, potentially from increased blood flow to the wings during exercise which could promote growth (Carrier and Leon, 1990), supporting previous work showing similar correlations between exercise excursions and body size in streaked shearwaters (Yoda *et al*., 2017). The absence of any direct relationship between wing extensions and wing growth might instead suggest chicks with longer wings are more likely to perform wing extensions (cf. wing extensions *causing* growth). However, previous work showed pre-fledging compensatory wing growth in the European starling (Allen *et al*., 2022), which could mean wing growth between days 15–20 is promoted by different mechanisms than earlier in development. Perhaps individuals that performed more wing extensions between days 16–20 also performed them at a higher rate before the beginning of our observations, promoting growth prior to day 15 and explaining the subsequent positive correlations between wing extensions and wing length. Neither exercise was associated with changes in mass, which does not support the prediction that increased activity levels are a mechanism for mass recession. Our data may therefore suggest that the mechanism for mass recession is not related to behaviour and instead may be related to physiological processes such as the metabolization of triglycerides (Allen *et al.* 2022) or water loss in maturing tissues (Ricklefs, 1968; O'Connor, 1977). In addition, our data do not rule out the possibility that pre-fledging exercises enable chicks to assess their body mass and facultatively adjust mass recession in response, as suggested by Wright *et al*. (2006), though we did not observe chicks doing "push-ups" like in common swifts (*Apus apus*).

Wing flapping is often interpreted in terms of motor skill acquisition, as chicks supposedly 'practice' for their first flight. In agreement, wing flapping increased significantly in the approach to fledging in European starlings and was unrelated to body size at fledging, suggesting it is unlikely to play a role in promoting growth. Instead, the daily mean number of wing flapping bouts was negatively associated with wing growth, suggesting increased wing flapping, but not wing extensions, may alternatively constrain growth. The surplus resource hypothesis predicts that play in juveniles, likely akin to exercise in some contexts, should only be favoured when surplus energy is available so that energy usage during motor skill acquisition does not subsequently limit growth (Graham and Burghardt, 2010). However, Assamese macaques (*Macaca assamensis*) were shown to trade-off motor skill acquisition, resulting from locomotor play, against growth under natural energy restriction (Berghänel *et al*., 2015), contradicting the surplus resource hypothesis. Our data partly support the findings of Berghänel *et al*. (2015), as there was correlative evidence of a potentially similar trade-off between exercise and growth here. Yet, the positive relationship between provisioning rate and wing flapping could still suggest that energy availability is an important factor influencing some exercise decisions in European starlings, agreeing with several studies in mammals that show how play often increases with greater food availability (Muller-Schwarze *et al*., 1982; Sharpe *et al*., 2002; Nunes *et al*., 2004; Cameron *et al*., 2008). Thus, we found mixed support for the surplus resource hypothesis, as wing flapping exercises that may promote motor skill acquisition were seemingly favoured with increased provisioning rates but might have still been performed at the expense of growth.

In conclusion, our results suggest that European starlings flap their wings and perform wing extensions in the approach to fledging, even without the ability to leave the nest to perform these exercises as has been observed in many larger birds in larger, open nests (Warham, 1990). Our data also provide some support for independent functions of these exercises, as they possessed separate relationships with chick age, morphology (wing growth and length), and food availability, although the correlative nature of this study makes it impossible to determine any causative effects. Therefore, future studies could experimentally manipulate pre-fledging exercises in birds, although researchers would need to consider how to restrict exercise without generally limiting movement, which would likely have unintended consequences on nestling development (e.g., begging, food acquisition, sibling competition). In addition, future studies could also explore physiological development in relation to pre-fledging exercise, as laboratory studies have shown the potential benefits of exercise for traits relating to flight ability, such as increased aerobic capacity (Yap *et al*., 2017). By further investigating exercise in the late stages of development, we might better understand the role of nestling behaviour in preparing for the workload transition at fledging and ultimately shed light on what influences individual variation in survival through this key life-stage.

4.6. Acknowledgements

We thank the Davis family for allowing us to use their farm as our field site and Alice Snell for managing equipment and collecting field data. We also thank Navkiran Poonia for her help with video analysis.

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Chapter 5.

Conclusions

This thesis tested traditional, and widely adopted, biological views relating to (morphological and physiological) metrics of juvenile quality and how those metrics might vary by developmental conditions and juvenile behaviour. We generally show that increased growth and body size does not always equal greater physiological maturity or improved physiological state, which should be considered when measuring juvenile quality (although this also depends on the underlying assumptions for using certain traits as metrics of quality; see below). Instrumental in this finding was that pre-fledging mass recession was seemingly a facultative process in European starlings, evidence of declines in mass being contextually adaptive. This presumably has knock-on effects for the relationships between juvenile body mass, physiologically state, and potentially post-fledging performance. Indeed, we provide experimental evidence that variations in body mass during pre-fledging development (due to weight-treatment in Chapter 2 and environmental variation in Chapter 3) might not *cause* any changes in physiological state in some species. Furthermore, body mass failed to reflect any metric of post-fledging performance (Chapter 2); however, we cannot say with any certainty here that this was *caused* by facultative mass recession. Nonetheless, the absence of relationships between body mass, physiological state, and post-fledging performance presented in this thesis, perhaps attributable to pre-fledging mass recession, highlights the importance of considering species-specific growth patterns when assessing juvenile quality and not assuming linear developmental trajectories.

It should also not be assumed that poor developmental conditions always result in poor developmental state in juveniles, as this thesis generally showed how many putative fitness-related traits (e.g., wing length, aerobic capacity) are robust to developmental context. Thus, certain morphological and physiological traits may be unreliable fitness biomarkers, as they fail to reflect environmental conditions that could be affecting other aspects of the juvenile phenotype or life-history. However, assessing physiological state in juveniles could help identify markers of poor developmental conditions, like here with oxidative status, as it reflected environmental context on several occasions, despite there being no contextual markers on structural growth or physiological maturation. We also showed how variation in the same environmental conditions (notably, provisioning rate) might predict variation in pre-fledging exercise, which could in-turn influence patterns of pre-fledging morphological development (wing growth). However, this contradicts previous chapters (1-3) and studies which suggest 'hardwired' or canalized development of wing growth. In addition, the minimal amount of time (<1% observed) dedicated to pre-fledging exercise might not be enough to cause variation in juvenile morphology, suggesting the observed relationships could be purely correlational. Thus, further work is required to test the causative and mechanistic relationships between pre-fledging exercise, environmental conditions, and wing development.

How then should we measure juvenile quality in birds? This is a question that my thesis sought to address, producing some results that are inconclusive and difficult to synthesize into a coherent answer. For example, body mass *was* a reliable marker of developmental conditions, depending on when it was measured (e.g., 15 days versus 20 days post-hatching; Chapters 1 and 3), while wing length never reflected developmental conditions. Perhaps this then suggests that body mass, but not wing length, is an appropriate metric of individual quality because it can reflect environmental context, which presumably affects some other measures of individual quality. Yet, canalization (Chapter 3) predicts that it is the most environmentally *insensitive* traits that should have the strongest links with fitness, which would then suggest that wing length, but not body mass, is an appropriate measure of individual quality. This paradox was also presented when measuring physiological state; reactive oxygen metabolites were a marker of developmental conditions (and the potential costs associated with them), while aerobic capacity varied little in response to environmental context. Should reactive oxygen metabolites then be used as a metric of individual quality because they reflect developmental conditions, or is that precisely why they should *not* be? Boonekamp *et al*. (2018) supports the latter; they showed that the physiological traits which varied most in response to environmental variation in jackdaws (*Coloeus monedula*) were the ones that had the weakest links with survival. In which case, this would suggest body mass and reactive oxygen metabolites are poor metrics of individual quality, as their sensitivity to developmental conditions predicts that they will be poorly related to fitness. Thus, if the traits that are most strongly related to fitness are the least variable, and the factors that determine the little variation they do have is uncertain, then another problem that remains at the end of this thesis is: what *determines* juvenile quality?

Implicit in answering the question of how to measure juvenile quality is the nature of the relationship between a developmental trait and fitness. Body mass, for example, has been discussed as a measure of individual quality for two (non-mutually exclusive) reasons: (1) it reflects developmental rate and/or physiological maturity, and (2) it reflects energy reserves. The former advocates for body mass as a measure of individual quality due to *indirect* relationships with fitness, that is, it is correlated with other (often less easily measured) physiological traits that are directly related to fitness; this is the main assumption that Chapters 1 and 2 disputes. The latter proposes body mass is a measure of individual quality because it is *directly* related to energy state (and Chapter 1 provides some evidence of this), which is then supposedly *directly* related to fitness (see Lack's energy reserves hypothesis, 1958), although we found no evidence of the latter (Chapter 2). The utility of a trait as a measure of individual quality is then likely determined by both direct and indirect links with fitness; if a trait is correlated with other fitness-related traits *and* is directly related to fitness itself, then that is likely a better measure of individual quality than a trait that only satisfies one or the other. Both these indirect and direct links should then be considered when we are interested in the strength of a relationship between a developmental trait and fitness.

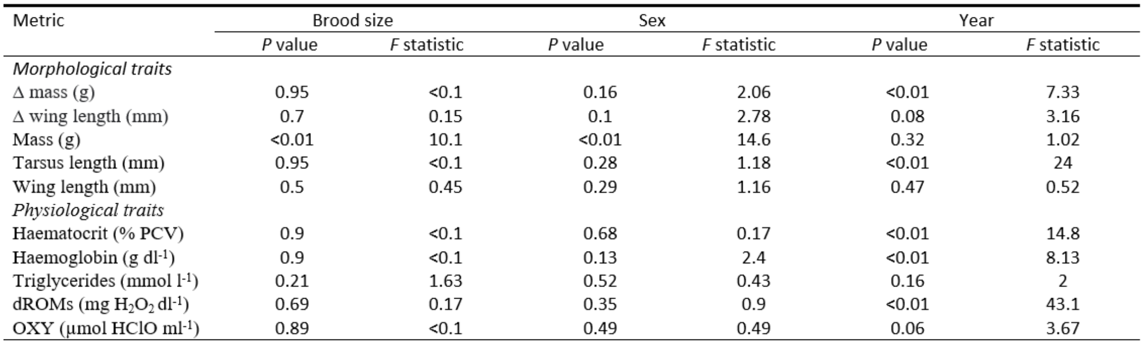
This thesis then does not argue that the use of some metrics of individual quality to date are inherently wrong, but instead attempts to explain the equivocal results relating to 'traditional', predicted relationships between body size and survival by considering *how* traits are related to fitness and, crucially, how these relationships might vary between species. Using our data, we can try to address the mixed results pertaining to juvenile body size and post-fledging survival in juvenile birds. In some species, body mass and physiological development are poorly correlated, potentially due to pre-fledging mass recession, negating the putative *indirect* relationships between body mass and fitness. In addition, there may also be cases where there are no *direct* links between body mass and fitness (although we are less confident in this interpretation, since we did not measure survival in Chapter 2), perhaps due to negative affects of greater body mass on flight performance and the adaptive significance of pre-fledging mass recession for optimizing wing-loading at fledging. In which case, body mass might be poorly correlated with juvenile survival in some species because it fails to reflect physiological state and does not directly benefit post-fledging performance. Equally, fledgling wing length has surprisingly failed to predict individual variation in post-fledging survival in some species (although it does appear to be more strongly associated with fitness than body mass). While we maintain the prediction that wing length is directly and mechanistically related to fitness (supported by correlations with post-fledging activity in Chapter 2), it may poorly reflect physiological state due to compensatory growth (Chapter 1) and/or canalized development (Chapter 3), negating any indirect relationships between wing length and fitness. In which case, this could make wing length a less reliable (but not unreliable) metric of juvenile quality and predictor of post-fledging survival.

This thesis then highlights a lot of uncertainty regarding both what determines individual variation in certain juvenile traits (since developmental conditions were surprisingly inconsequential) and how variation in these traits relates to juvenile fitness (perhaps due to poor links with physiological state). Our work suggests that both the predicted indirect and direct relationships between body size and fitness might be flawed assumptions in juveniles of some bird species. Pre-fledging development (both flexible and inflexible trajectories) might partly explain these absent relationships, in which case we encourage future work to consider *when* to assess juvenile quality during development. Indeed, Chapter 1 suggested that day 15 trait values might better predict physiological state at fledging than those same values at day 20. When to measure individual quality should also be considered in conjunction with species-specific developmental patterns, as variable growth trajectories may decouple or weaken predicted relationships between body size versus physiological state and/or post-fledging performance. Variation in growth trajectories may then be more common during certain developmental stages, like in the approach to the work-load transition at fledging, and this is something that should be considered in future studies. However, what determines individual variation in these growth trajectories and physiological state is still uncertain; we encourage future work investigating differences in developmental stage (e.g., are future canalized developmental patterns pre-determined during early development?) and the role of parental quality (e.g., heritability) in dictating whether their offsprings' canalized development will be 'set' towards becoming 'good' or 'bad' quality juveniles.

Appendix A.

Supplemental tables and figures from Chapter 1

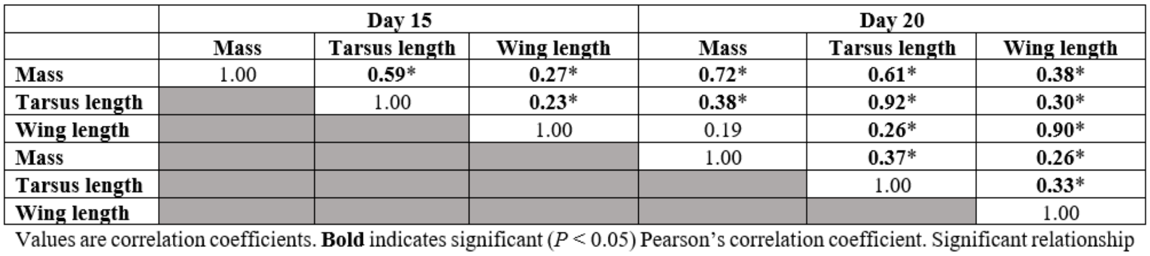
Table A.1 **Results from linear mixed-effects models (LMMs) of morphological and physiological development in nestling European starlings (*Sturnus vulgaris*) by ecological context (brood size and year; *n* = 89) and sex (for chicks in 2021 only; *n* = 43). Chicks were measured in 2020 and 2021 in British Columbia, Canada, starting 15 days after hatching and ending on day 20, one day prior to typical fledging age.**



Metric	Brood size		Sex		Year	
	P value	*F* statistic	*P* value	*F* statistic	*P* value	*F* statistic
Morphological traits						
Δ mass (g)	0.95	<0.1	0.16	2.06	<0.01	7.33
Δ wing length (mm)	0.7	0.15	0.1	2.78	0.08	3.16
Mass (g)	<0.01	10.1	<0.01	14.6	0.32	1.02
Tarsus length (mm)	0.95	<0.1	0.28	1.18	<0.01	24
Wing length (mm)	0.5	0.45	0.29	1.16	0.47	0.52
Physiological traits						
Haematocrit (% PCV)	0.9	<0.1	0.68	0.17	<0.01	14.8
Haemoglobin (g dl^{-1})	0.9	<0.1	0.13	2.4	<0.01	8.13
Triglycerides (mmol l^{-1})	0.21	1.63	0.52	0.43	0.16	2
dROMs (mg H_2O_2 dl^{-1})	0.69	0.17	0.35	0.9	<0.01	43.1
OXY (μmol HClO ml^{-1})	0.89	<0.1	0.49	0.49	0.06	3.67

dROMs, reactive oxygen metabolites; OXY, antioxidant titers. Δ denotes the change in a trait between days 15-20 after hatching. All other metrics pertain to day 20 after hatching only. LMMs included nest ID as a random factor. Values for triglycerides and dROMs were logarithmically transformed.

Table A.2 **Correlation matrix for morphological traits in European starling nestlings on day 15 (*n* = 98) and 20 (*n* = 89) after hatching, one day prior to typical fledging age. Chicks were measured in 2020 and 2021 in British Columbia, Canada.**



	Day 15			Day 20		
	Mass	**Tarsus length**	**Wing length**	**Mass**	**Tarsus length**	**Wing length**
Mass	1.00	**0.59***	**0.27***	**0.72***	**0.61***	**0.38***
Tarsus length		1.00	**0.23***	**0.38***	**0.92***	**0.30***
Wing length			1.00	0.19	**0.26***	**0.90***
Mass				1.00	**0.37***	**0.26***
Tarsus length					1.00	**0.33***
Wing length						1.00

Values are correlation coefficients. **Bold** indicates significant ($P < 0.05$) Pearson's correlation coefficient. Significant relationship within linear mixed effects model (LMM) indicated by *. The results of LMMs informed covariate selection of models reported in the main text. Nest ID was used as a random factor. Year was included as a covariate in all models, whereas brood size was only included as a covariate in models involving mass.

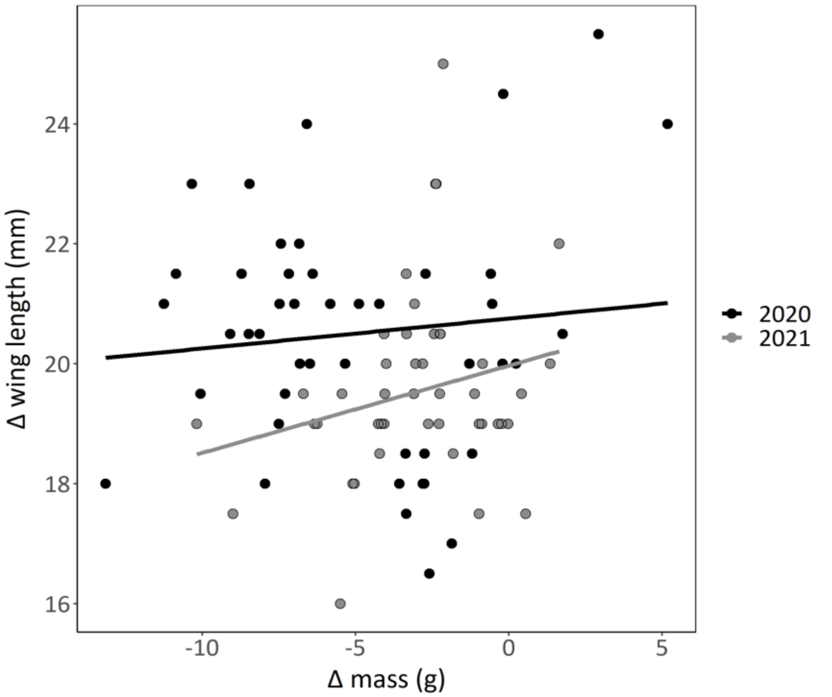


Figure A.1 **Individual variation in the change (Δ) in mass between 15–20 days after hatching in nestling European starlings versus Δ wing length. Development of these traits were unrelated (*P* = 0.14, $F_{1,84}$ = 2.22). This analysis was performed using a linear mixed effects model, including year and brood size as covariates, along with nest ID as a random factor.**

Table A.3 **Correlation matrix for physiological traits in nestling European starlings (*n* = 89) on day 20, one day prior to typical fledging age (i.e., 20 days after hatching). Chicks were blood sampled in 2020 and 2021 in British Columbia, Canada.**

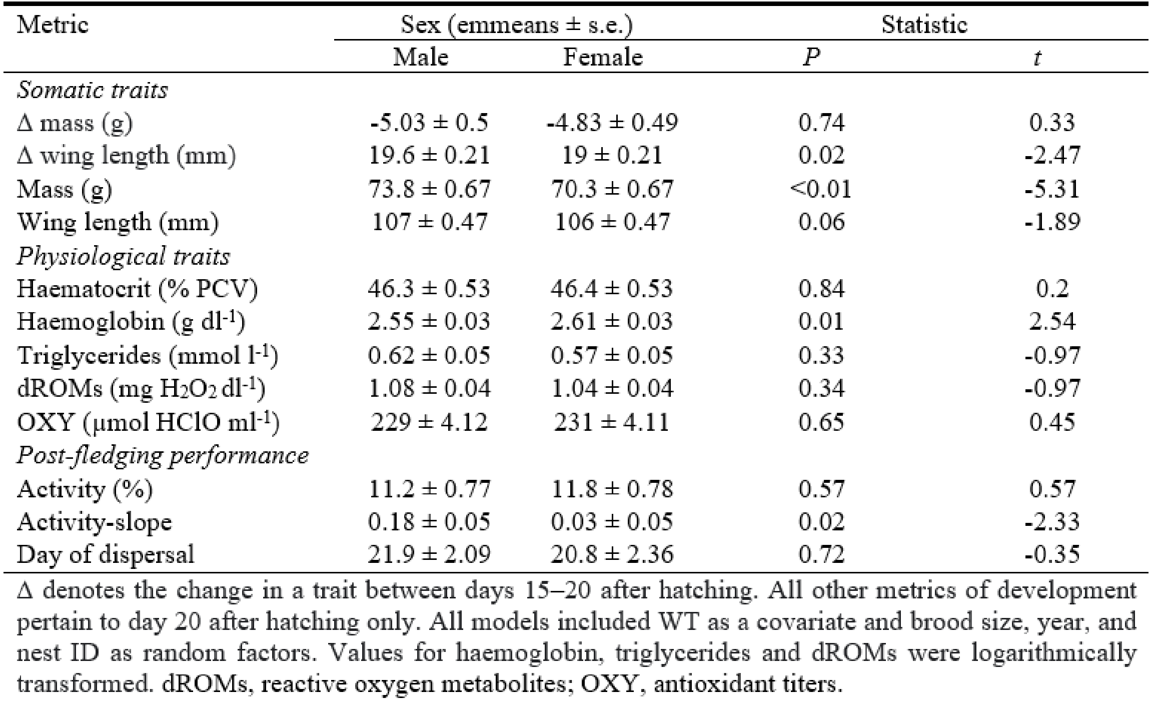
	Haematocrit	**Haemoglobin**	**Triglycerides**	**dROMs**	**OXY**
Haematocrit	1.00	**0.62***	**0.30**	0.19	0.16*
Haemoglobin		1.00	**0.27**	0.14	**0.26***
Triglycerides			1.00	**0.36***	**0.23***
dROMs				1.00	0.08*
OXY					1.00

Values are correlation coefficients. **Bold** indicates significant (*P*<0.05) Pearson's correlation coefficient. Significant relationship within linear mixed model (LMM) indicated by *. The results of LMMs informed covariate selection of models reported in the main text. Nest ID was used as a random factor and year was used as a covariate for all models except triglycerides ~ OXY.

Appendix B.

Supplemental tables and figures from Chapter 2

Table B.1 **Estimated marginal means (emmeans) and paired contrast results of variation in somatic and physiological development, as well as post-fledging performance, by sex in European starlings (*Sturnus vulgaris*). Chicks were measured in British Columbia, Canada, between 2020–2022, starting at 15 days after hatching and ending on day 20, one day prior to typical fledging age. Post-fledging performance pertains to data from 2021–2022 only.**



Metric	Sex (emmeans ± s.e.)		Statistic	
	Male	Female	*P*	*t*
Somatic traits				
Δ mass (g)	-5.03 ± 0.5	-4.83 ± 0.49	0.74	0.33
Δ wing length (mm)	19.6 ± 0.21	19 ± 0.21	0.02	-2.47
Mass (g)	73.8 ± 0.67	70.3 ± 0.67	<0.01	-5.31
Wing length (mm)	107 ± 0.47	106 ± 0.47	0.06	-1.89
Physiological traits				
Haematocrit (% PCV)	46.3 ± 0.53	46.4 ± 0.53	0.84	0.2
Haemoglobin (g dl^{-1})	2.55 ± 0.03	2.61 ± 0.03	0.01	2.54
Triglycerides (mmol l^{-1})	0.62 ± 0.05	0.57 ± 0.05	0.33	-0.97
dROMs (mg H_2O_2 dl^{-1})	1.08 ± 0.04	1.04 ± 0.04	0.34	-0.97
OXY (μmol HClO ml^{-1})	229 ± 4.12	231 ± 4.11	0.65	0.45
Post-fledging performance				
Activity (%)	11.2 ± 0.77	11.8 ± 0.78	0.57	0.57
Activity-slope	0.18 ± 0.05	0.03 ± 0.05	0.02	-2.33
Day of dispersal	21.9 ± 2.09	20.8 ± 2.36	0.72	-0.35

Δ denotes the change in a trait between days 15–20 after hatching. All other metrics of development pertain to day 20 after hatching only. All models included WT as a covariate and brood size, year, and nest ID as random factors. Values for haemoglobin, triglycerides and dROMs were logarithmically transformed. dROMs, reactive oxygen metabolites; OXY, antioxidant titers.

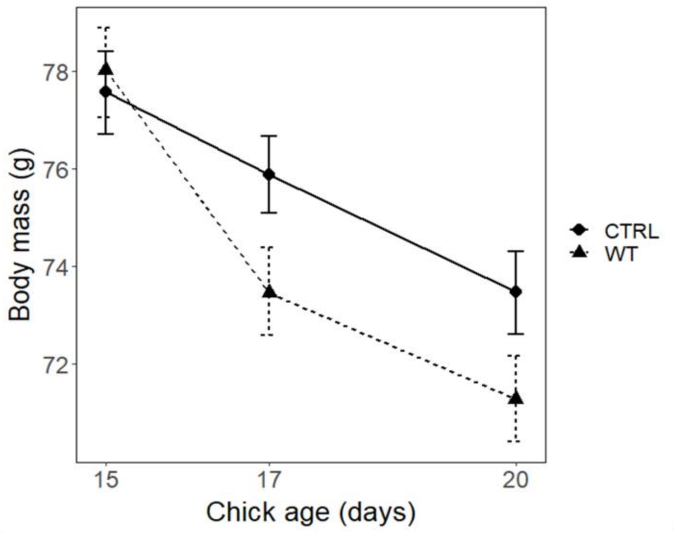


Figure B.1 **Body mass approaching fledging in control and weight treated European starling nestlings. Data is from all years (2020–2022). There was a significant WT × age interaction; changes in mass between groups were only significantly different between ages 15–17, while mass**

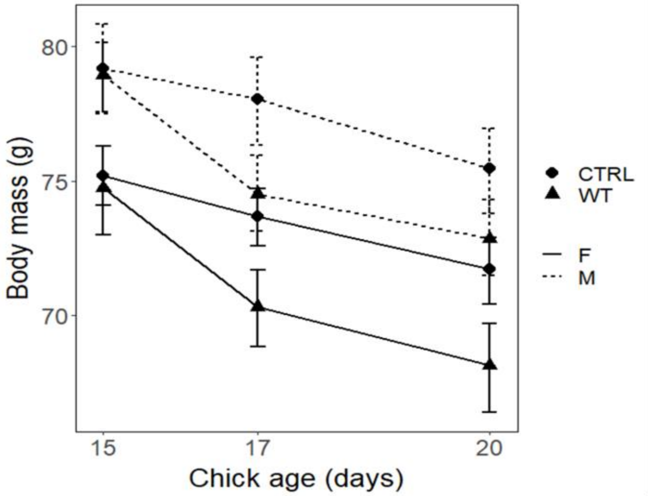


Figure B.2 Body mass by sex approaching fledging in control and weight treated European starling nestlings. Data is from two years only (2021–2022). There was a significant WT × age interaction; changes in mass between groups were only significantly different between ages 15–17, while mass trajectories between ages 17–20 were equivalent. Body mass significantly

Example handling of sex data. Since body mass significantly varies by sex (Table B.1), we ran our Mass~Age*WT model using 2021–2022 data only and first used AIC to test whether including sex as an interaction term improved the model. AIC was lower when including sex as a covariate (cf. interaction term), since there was no significant age × sex, WT × sex, or age × WT × sex interactions (figure S2; $p < 0.43$ in all cases), proving a consistent response to treatment regardless of sex (i.e., changes in mass and the timing of changes in mass resulting from WT did not differ between males and females). We therefore ran the model including sex as a covariate using only the 2021–22 data, after which we ran the model again but excluding sex and including all available data. In both cases, using all data available and not including sex as a covariate, or using only 2021–2022 data and including sex as a covariate, resulted in equivalent age x WT interactions and mean changes in mass between treatments. Consequently, we reported the results of the model not including sex in the main text (figure S1), as to include all available data. If AIC supported the inclusion of sex as an interaction and the interaction was significant, or if including sex as a covariate significantly changed the results of a model, then only data from 2021–2022 including sex would be reported in the main text.

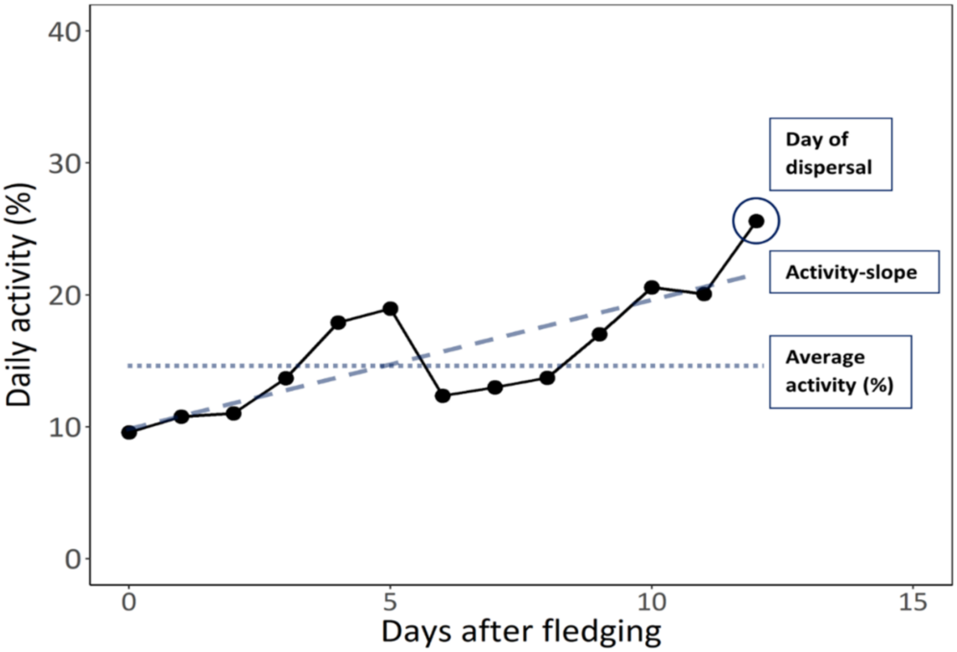


Figure B.3 Metrics of post-fledging performance. Example data from a single radiotransmitter fitted to a European Starling chick 20 days after hatching in British Columbia, Canada. Daily activity represents marginal means for activity that were calculated using LMMs with the ratio of active to inactive detections, days post-fledge, and number of daily detections as main effects.

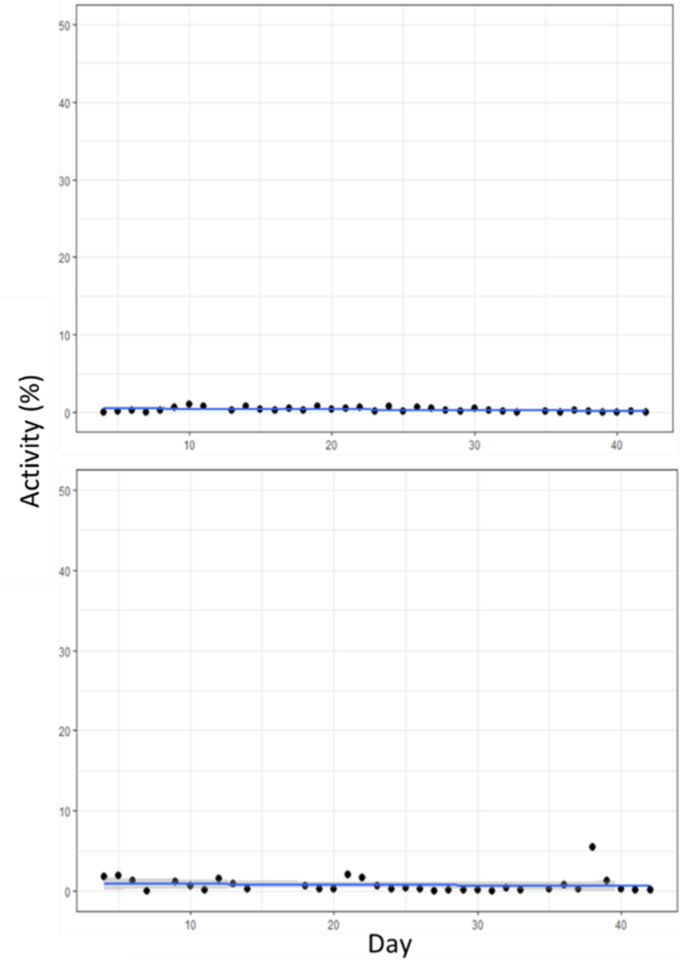


Figure B.4 **Stationary tag validation. Activity of two stationary radiotransmitters placed in separate locations about the field site in 2018. Stationary tags were deployed alongside tagged European starling fledglings (n = 28).**

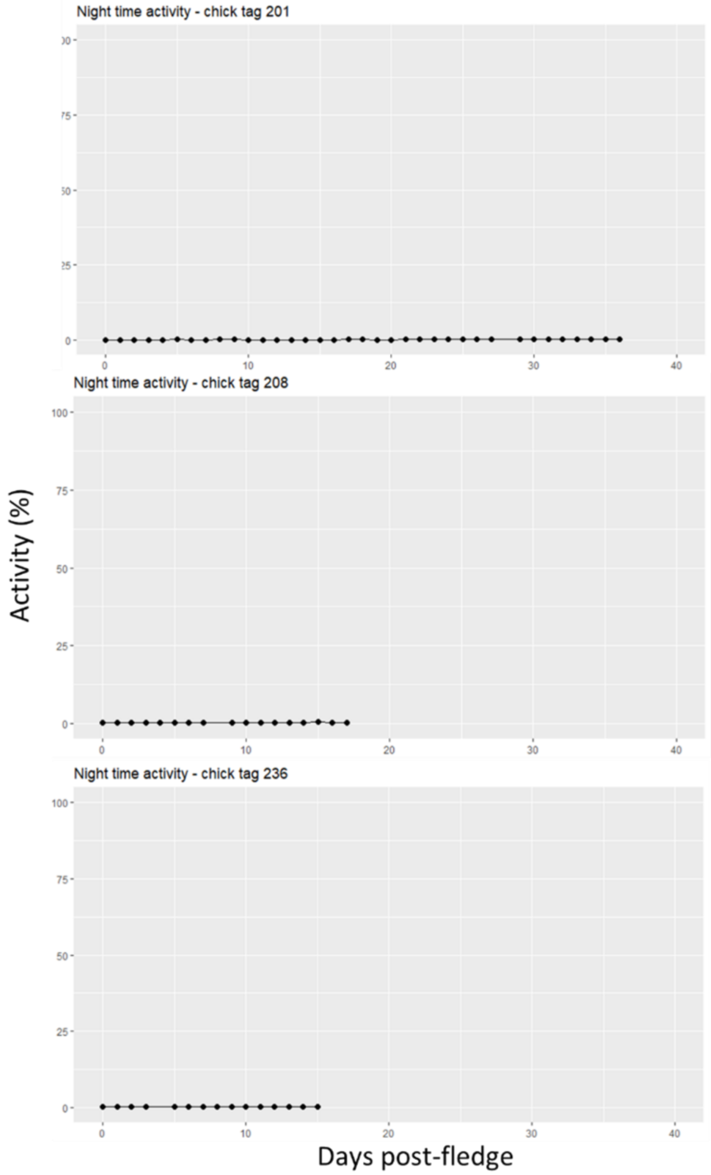


Figure B.5 **Night time validation. Example European starling activity at night time from three radiotransmitters (*n* = 28 total) fitted to European starling fledglings in 2018.**

Table B.2 Correlation matrix for metrics of post-fledging performance in fledgling European starlings. Radiotransmitters (*n* = 89) were fitted to fitted to chicks 20 days after hatching between 2021–2022 in British Columbia, Canada.

	Activity	**Activity-slope**	**Day of dispersal**
Activity	1.00	0.08	-0.26
Activity-slope		1.00	-0.25
Day of dispersal			1.00

Values are correlation coefficients. **Bold** indicates significant ($P<0.05$) Pearson's correlation coefficient. Significant relationship within linear mixed model (LMM) indicated by *.

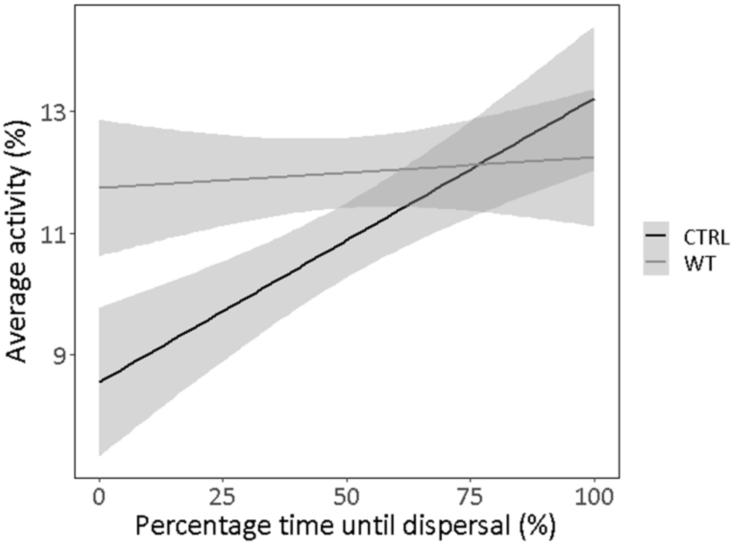


Figure B.6 Average activity between fledging and dispersal by treatment. Data is from *n* = 74 European starling fledglings that displayed more than five days of activity and had showed no evidence of mortality. Controls demonstrated significantly greater activity-slopes than WT chicks, driven by lower activity immediately after fledging (first 10 days) in controls but subsequent increases in activity between fledging and dispersal, compared to WT chicks that were immediately more active after fledging but showed little increase in activity between fledging and dispersal. There was no difference in average activity across the entire period (see "Results").

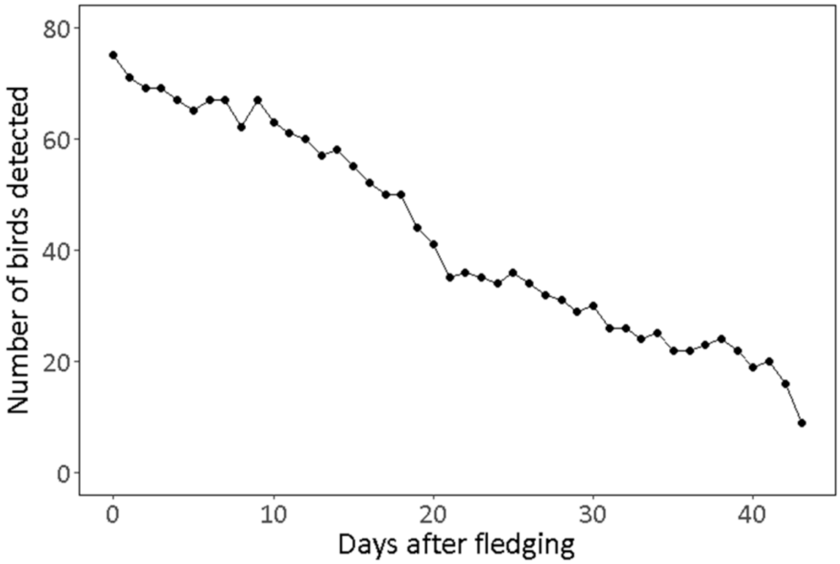


Figure B.7 **Timing of dispersal in European starlings. Data is from *n* = 82 European starling fledglings that had showed no evidence of mortality. Dispersal was defined as the last day of more than 100 detections for an individual radiotransmitter. Most fledglings (*n* = 68) dispersed prior to the final day of observation, while 14 chicks showed no evidence of dispersal. There was no difference in day of dispersal between WT and control groups (see "Results").**

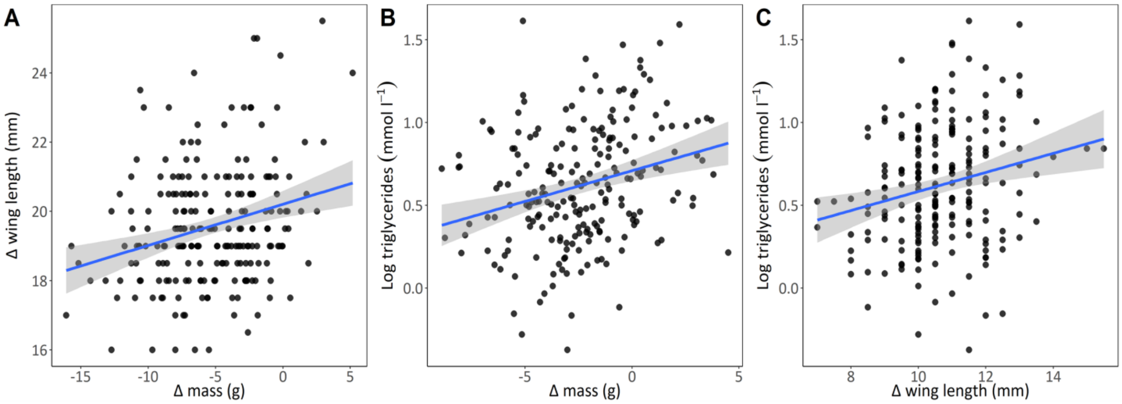


Figure B.8 **Correlated somatic trajectories in relation to energy state. Δ mass vs Δ wing length between days 15–20 (A), Δ mass between days 17–20 vs plasma triglycerides at day 20 (B), and Δ wing length between days 17–20 vs plasma triglycerides at day 20 (C). In (A), there was a significant positive relationship ($p < 0.01$, $F_{1,210} = 12.6$) and no WT interaction ($p = 0.41$, $F_{1,177} = 9.67$). In (B) there was a significant positive relationship while controlling for Δ wing length ($p < 0.01$, $F_{1,207} = 16.8$) and there was a significant positive relationship in (C) while controlling for Δ mass ($p = 0.02$, $F_{1,200} = 5.9$); neither demonstrated a significant WT interaction ($p > 0.08$ in both cases).**

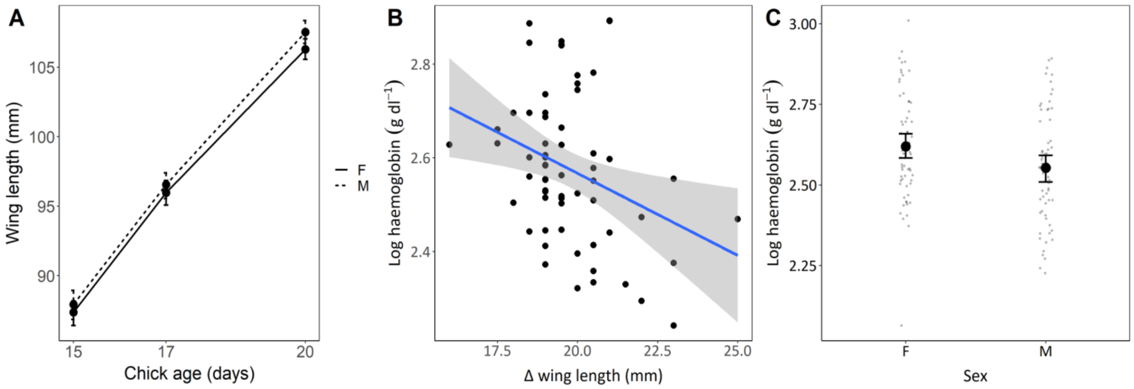


Figure B.9 Lower haemoglobin in male European starling fledglings associated with accelerated wing growth. Wing length approaching fledging in controls by sex (A), ∆ wing length vs haemoglobin in controls (B), and fledgling haemoglobin by sex (C). In (A), there was a significant age × sex interaction in ($p < 0.01$, $F_{1,258} = 14.4$). While wing growth showed no sex difference between ages 15–17 ($p = 0.58$, $t_{1,256} = 0.55$), male wing growth was significantly greater than females' between ages 17–20 ($p < 0.01$, $t_{1,256} = 4.29$). In (B), there was a significant negative relationship between ∆ wing length and haemoglobin ($p < 0.01$, $F_{1,53} = 9.23$) and no sex interaction ($p = 0.47$, $F_{1,58} = 0.52$). In (C), there was a significant difference between male and female haemoglobin at fledging ($p = 0.01$, $t_{1,109} = 2.56$). Together, these results suggest males accelerate wing growth approaching fledging, but this comes as at a physiological cost of reduced haemoglobin. Data is from 2021–2022 only, as sexing data was not available for 2020.

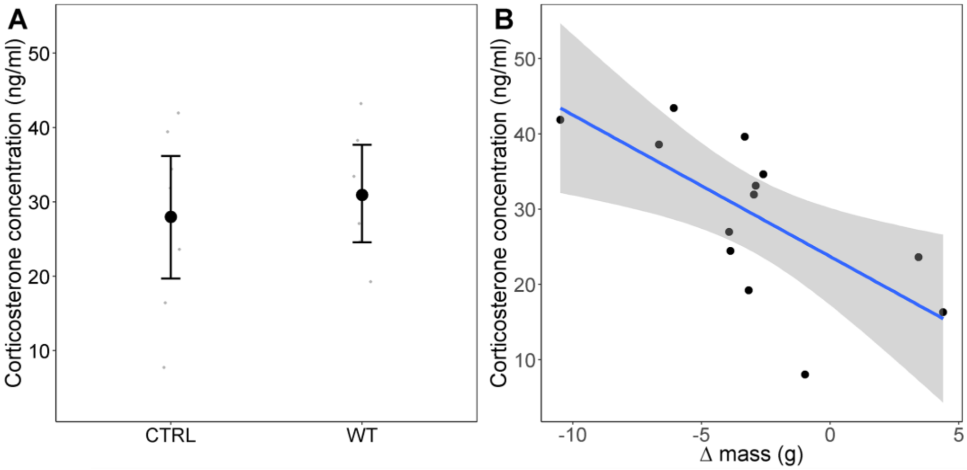


Figure B.10 **Plasma corticosterone concentrations by treatment and in relation to Δ mass. Corticosterone concentrations by treatment (A), and corticosterone concentrations vs Δ mass (B). Data is from *n* = 13 European starling nestlings blood sampled 17 days after hatching immediately following the period of experimentally increased mass recession resulting from weight-treatment (i.e., days 15–17). These samples comprised the only individuals with handling times less than 3 minutes from *n* = 40 chicks that were blood sampled on day 17 in 2021. In (A), there was no significant difference between groups (p = 0.63, $t_{1,11}$ = -0.49). In (B), the relationship was significant (p = 0.01, $t_{1,11}$ = -3.07). Δ mass pertains to changes in mass between days 15–17 only. Mean mass change was -4.43 ± 1.56 g in WT chicks (*n* = 6) and -1.79 ± 1.56 g in controls (*n* = 7). Samples were assayed in duplicate (intra-assay CV: 1.84%) using an ELISA kit (ADI-900-097, Enzo Life Sciences) and following the manufacturer's instructions.**