

RESEARCH ARTICLE

Variation in developmental trajectories of physiological and somatic traits in a common songbird approaching fledging

Allison Cornell* and Tony D. Williams

ABSTRACT

In avian species, little is known about the development of physiological traits in the days preceding fledging, a critical life history transition marked by a high mortality rate. Developmental trajectory during this period may be flexible based on ecological context or hardwired, with potential costs for variation in growth in the form of oxidative stress. Patterns in development are likely to relate to variation in life history, for which seabirds and aerial insectivores have been well studied, while our focal species is a grassland ground forager, the European starling (*Sturnus vulgaris*). We show that changes in haematocrit, body mass and wing length are independent of year and brood quality, while changes in haemoglobin concentration are higher in low-quality broods. Moreover, we also identify higher oxidative stress in low-quality year and second broods, a potential cost for maintaining a hardwired developmental trajectory in a lower quality environment. Finally, we experimentally test the effects of food supplementation on development and maturity of chicks at fledging to show that although food increases body mass early in development, it does not change the trajectory or final maturity of chicks at fledging. Collectively this study demonstrates that some developmental changes prior to fledging may be hardwired, but may have long-term oxidative costs in low-quality environments.

KEY WORDS: Development, Chick, Ecophysiology, Food supplementation, Haemoglobin, Haematocrit

INTRODUCTION

Variation in developmental trajectories in avian chicks has been widely studied, especially across broad taxonomic divisions, e.g. in relation to major patterns of developmental mode such as the altricial-precocial spectrum (Ricklefs, 1979; Starck, 1998; Blom and Lilja, 2005). Much of this work has focused on embryonic development and maturity at hatching (Ricklefs and Starck, 1998; Tazawa, 2005) or transitions associated with hatching itself, e.g. the change from respiration via diffusion to convection in the perinatal period (Vleck and Bucher, 1998). In general there has been less work on developmental trajectories approaching another major ‘developmental milestone’, the transition at fledging from a sedentary nestling to the fully volant, active lifestyle of post-fledging juveniles. Furthermore, much work on intraspecific variation in patterns of offspring development has focused on growth measured as mass (Ricklefs and Starck, 1998) or functional maturity of major tissues (e.g. Vézina et al., 2009; but see Vleck and

Bucher, 1998). Less work has focused on intraspecific variation in development of physiological traits, in particular traits that might have significant fitness effects in the immediate post-fledging period, e.g. oxygen carrying capacity (Gebhardt-Henrich and Richner, 1998; Bowers et al., 2014; Cornell et al., 2016a,b).

Variation in developmental trajectories has been related to developmental mode, life history variation of different taxa (e.g. seabirds), and in particular requirements for locomotion and flight ability immediately post-fledging (Ricklefs, 1973; Murton and Westwood, 1977). Consistent with this finding of variation in development in relation to life history differences, we predict that the ground foraging European starling will show a different pattern of development than previously studied aerial insectivore species (Simmons and Lill, 2006) based on their differing life history patterns. Ricklefs and Starck (1998) proposed that variation in developmental trajectories of chicks, such as the timing and magnitude of asymptotic mass gain, might be explained by three non-mutually exclusive levels of constraint: (1) availability of food resources to chicks, which poses an ecological limit to growth rate; (2) limitations in the individual chick’s capacity to utilize available resources, e.g. the capacity of the gut to assimilate food; or (3) limitations arising from ‘a basic antagonism at the tissue or cell level between chick and adult function’. In the latter case, for example, before fledging sedentary chicks do not have a requirement for high levels of aerobic capacity, or metabolic rate, but these are needed at the point of fledging. While it has been suggested that these constraints might have differential effects on growth in size of chicks (i.e. somatic components) versus physiological maturation (i.e. changes in tissues and organs that approach ‘adult level of function’; Ricklefs and Starck, 1998) few experimental studies have directly compared development rate of different traits to attempt to determine the relative importance of external (i.e. ecological) or internal constraints. We hypothesize that there will be variation in the developmental trajectories of somatic and physiological traits across seasonally and annually varying ecological conditions.

Here we investigate intraspecific variation in developmental trajectories just before fledging for somatic (mass, wing length) and physiological traits (haematocrit, haemoglobin concentration) in European starling chicks, to test how somatic and physiological development may be affected by predicted variation in resources. Specifically, we compare variation in rates of development in different ecological contexts: (a) among years of different reproductive productivity, and (b) in chicks from first and second broods, as generally second brood chicks develop during periods of lower seasonal food availability, breeding success is lower, and second brood chicks fledge at lighter mass (Cornell et al., 2016a,b) and have lower post-fledging survival (Verboven and Visser, 1998; Naef-Daenzer et al., 2001). As there can be costs of higher growth rates we also measured reactive oxygen metabolites and anti-oxidant capacity to test if annual, brood, or individual variation in developmental trajectories prior to fledging were correlated with

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6.

*Author for correspondence (aecornell@email.wm.edu)

 A.C., 0000-0002-8782-084X

Received 2 May 2017; Accepted 8 September 2017

levels of oxidative stress at fledging. Finally, we experimentally manipulated growth conditions with supplemental feeding and tested for differential responses in terms of changes in developmental trajectories for somatic and physiological traits, and associated effects on oxidative stress.

MATERIALS AND METHODS

Field and laboratory methods

We measured variation in chick physiological and somatic development just before (day 17) and at fledging (day 21) in European starlings (*Sturnus vulgaris* Linnaeus 1758) at Davistead Farm, Langley, British Columbia, Canada (49.17°N, 122.83°W). All protocols were approved by Simon Fraser University Animal Care Committee (UACC) permits 1074B-94 and 1172B-96. Our dataset includes 2 years of differing productivity: in 2013, 2.4±1.8 chicks fledged per nest ($n=54$ nests) from first broods; in 2014, 3.7±1.7 chicks fledged ($n=31$ nests; unpaired t -test $P=0.03$); long-term average 3.01 chicks ($n=15$ years). The difference in sample size between years was due to the number of experimental treatments on the site in 2014 (radiotransmitters and wing clipping of parents) and treated nests were not included in the dataset. The difference in chicks surviving to fledge between 2013 and 2014 is related to chick mortality during the nestling stage, as there was no difference in clutch size ($P=0.25$, unpaired t -test) or brood size at hatch ($P=0.76$, unpaired t -test) between the two years. We also have one experimental year (2015, see below). This field site includes ca 150 nest boxes mounted on posts around pastures and on farm buildings, and houses ~70 breeding pairs annually. Nest boxes were checked daily beginning in late March to determine the lay date of the first egg and clutch size. Nests were monitored until either failure or fledging to quantify productivity. On the seventeenth day after hatching, all chicks were removed from each nest and measured for mass using a digital scale to 0.01 g, tarsus using digital callipers, and wing length, and blood samples were taken from the left wing's brachial vein for measurement of haematocrit and haemoglobin concentration. Four days after the typical fledging date (day 21 post-hatching), two random chicks (excluding runt chicks) were removed from each nest box for second, repeated measurements of mass and wing length, and a blood sample was taken from the right wing's brachial vein for physiological measurement (repeated measurement of haematocrit and haemoglobin concentration, and one-time measurements of oxidative stress; see below). In 2014 we obtained detailed information on age of fledging: the majority of chicks (106/111, 95%) fledged on day 21 post-hatching with only 5/111 chicks fledging on day 20, i.e. before we removed birds for sampling. Birds with only day 17 measurements were not included in analyses. After the fledging of first broods, full data collection was repeated, as described above, for second broods.

Haematocrit was measured with digital calipers (± 0.01 mm) following centrifugation of whole blood for 3 min at 13,000 g (Microspin 24; Vulcon Technologies, Grandview, MO, USA). Haemoglobin concentration (g dl^{-1} whole blood) 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), using 5 μl whole blood diluted in 1.25 ml Drabkin's reagent (D5941; SigmaAldrich Canada, Oakville, Ontario, Canada) with absorbance measured at 540 nm. We ran all haemoglobin samples in duplicate to calculate coefficient of variation (0.7%) as a measure of intra-assay variation, and a single pooled sample each year across all plates to calculate average inter-assay coefficient of variation (1.6%). An aliquot of these samples were sent to Viaguard Accu-

Metrics (Toronto, Ontario, Canada) for sexing by polymerase chain reaction (PCR), along with known samples (from adults) for quality control. We measured oxidative stress following methods described in Tissier et al. (2014) using d-ROMs and OXY tests (Diacron International, Grosseto, Tuscany, Italy) to test reactive oxygen metabolites and total anti-oxidant capacity of the plasma, respectively. We examined these metrics independently and as a ratio, following Costantini et al. (2007). We ran all samples in duplicate to calculate coefficient of variation (OXY: 5.1%; d-ROMs: 6.4%) as a measure of intra-assay variation. To determine inter-assay variation we used a single pooled sample each year across all plates to calculate average inter-assay coefficient of variation (OXY: 5.9%; d-ROMs: 11.6%).

Supplemental feeding experiment

During 2015 we conducted a supplemental feeding experiment with both first and second broods. In each nest we evenly distributed chicks between experimental and control groups (i.e. $n=2$ fed chicks and $n=2$ control chicks in brood size of four) to control for nest or common parental effects. Initially, due to concerns about potential negative effects of disturbance on young chicks we started supplemental feeding at day 10 post-hatching for first broods. However, we were subsequently able to start feeding chicks at day 4 post-hatching for most first broods and all second broods. We therefore had the following treatments: (a) chicks fed since day 4 ($n=20$ chicks, 12 nests) and their respective controls ($n=20$ chicks, 12 nests) in first broods, (b) chicks fed since day 10 ($n=16$ chicks, eight nests) and their respective controls ($n=16$ chicks, eight nests) in first broods, (c) chicks fed since day 4 ($n=23$ chicks, 15 nests) and their respective controls ($n=25$ chicks, 16 nests) in second broods. Chicks were weighed at the end of each day (after supplementation) to monitor body mass and fed chicks received supplemental food twice a day with the daily total amount of food per day (summing both meals) equivalent to 10% of predicted daily mass gain in European starling chicks as reported in Westerterp et al. (1982). We stopped daily supplemental feeding and mass measurements on day 18 to prevent force-fledging, but did collect measurements at fledging (day 21) as described above. The supplemental food diet was the same as reported in Eng et al. (2014), which is similar in protein content (11.9%) to the most common starling prey item (12.8%), Tipulid larvae (Brodmann and Reyer, 1999). Begging behavior during feeding visits was stimulated by the researcher making high-pitched vocalizations. Control chicks were weighed and handled equally to fed chicks, but without supplemental feeding. We evenly distributed hatching order across fed and control groups, although any runt chicks defined as hatching >24 h after first-hatched chicks within nests (8% of all chicks) were excluded from the analysis.

Statistical analysis

All analyses were completed in RStudio version 0.99.902 (RStudio Team, 2015) using lsmmeans (Lenth, 2016), nlme (R package version 3.1-128, <https://CRAN.R-project.org/package=nlme>), and stats (RStudio Team, 2015) packages. In the text portion of the results, means are presented as \pm standard deviation.

To compare the slopes of developmental trajectories just prior to fledging we calculated development expressed as a percentage change between days 17 and 21 by subtracting the day 17 measurement from the day 21 value, and dividing by the original day 17 value to control for differences in starting maturity of the chicks. We ran linear mixed effects models on the per cent change values, controlling for parent identity (ID) as a random factor, and

with brood number, sex and year as main effects with their interactions. We used the same models to determine the relationship between mass change from day 17 to day 21 (raw values) related to change in wing length, haemoglobin concentration or haematocrit (raw values). In this model, mass change was included as a main effect and as an interaction with all possible combinations of brood, year and sex, with parent ID as a random factor.

In order to determine the effects of year and brood on the developmental change between days 17 and 21, we ran linear mixed effects models with age (day 17 or day 21), brood, year and sex as main effects, and all possible interactions. Parent ID was included as a random factor. Using this model, interactions between age and year, or age and brood represent effects of year or brood on the rate of developmental change (slope) between days 17 and 21. For each oxidative stress metric (anti-oxidant capacity, reactive oxygen metabolites, ratio of reactive oxygen metabolites to anti-oxidants) we used the same linear mixed effects model, but without the age term (these measurements were only made once). For experimental data, we used the same original model (including the age term) with treatment group included as a main effect and in interactions, and year removed (as experimental data was only collected in one year). In 2013 among first broods, 25 chicks were female and 35 were male, among second broods eight were female and six were male. In 2014, 11 chicks from first broods were female and 16 were male, among second broods nine were female and five were male.

In order to determine how oxidative stress relates to developmental change, we calculated the per cent change between days 17 and 21 (described above) in a linear mixed effects model with each oxidative stress metric as an explanatory factor in a model of oxidative stress (each metric in its own model). The model controlled for year, brood, sex and interactions as covariates and parent ID as a random factor.

Data for this paper are available in Dryad (Cornell et al., 2016a,b).

RESULTS

Developmental trajectories prior to fledging

All somatic and physiological traits changed significantly over the 4 days prior to fledging, between 17 and 21 days post-hatching (age effect, Table 1). However, whereas wing length, haematocrit and haemoglobin increased, by 16.8, 11.3 and 10.8% respectively, body mass decreased (by 5.2%) over the same time period (Fig. 1). Individual variation in the decrease in mass was not significantly related to variation in the change of haemoglobin ($P=0.47$, $F_{1,85}=0.5$) or haematocrit ($P=0.28$, $F_{1,97}=1.2$), but was related to change in wing length ($P<0.05$, $F_{1,99}=4.0$).

Body mass was unrelated to sex and almost all other combinations of interactions in the full model (Table 1). Furthermore, age×brood and age×year interactions were not significant for body mass ($P>0.3$; Table 1), i.e. developmental trajectories did not vary among broods or years. The brood×year interaction was significant in the full model ($P=0.02$, $F_{1,213}=5.3$): chicks from 2013 (75.59 ± 7.01 g) had 0.9% higher day 17 mass than 2014 chicks (74.91 ± 4.59 g) in first broods ($P=0.04$, $F_{1,75}=4.2$, controlling for sex and interactions). This difference was not significant when first brood chicks were 21 days old ($P=0.10$, $F_{1,68}=2.8$). There was no difference in mass at 17 or 21 days across years in second broods (day 17: $P=0.21$, $F_{1,12}=1.8$; day 21: $P=0.11$, $F_{1,12}=3.1$). First brood chicks at day 17 had 5.6% higher mass than second broods, and 4.6% higher than second broods at day 21 (Table 1). Age×year×brood was not significant in the full model ($P=0.98$, $F_{1,169}<0.1$).

Wing length showed no significant age×brood or age×year interactions ($P>0.2$; Table 1). However, there was a significant

effect of sex (Table 1), sex×brood ($P=0.02$, $F_{1,189}=5.7$) and sex×year interactions ($P=0.04$, $F_{1,183}=4.2$). In second broods, day 21 females had 3.6% shorter wings than males (98.2 ± 4.0 versus 101.9 ± 5.5 mm; $P=0.04$, $F_{1,17}=5.1$, controlling for year and interactions) but not in first broods ($P=0.41$, $F_{1,63}=0.7$) or on day 17 ($P=0.17$, $F_{1,20}=2.1$). In 2014, male chicks had longer wings (89.7 ± 6.0 mm) than females (85.2 ± 6.0 mm) on day 17 ($P=0.03$, $F_{1,30}=5.0$ controlling for brood and interactions), but not on day 21 ($P=0.07$, $F_{1,29}=3.6$) or in 2013 (day 17: $P=0.88$, $F_{1,45}<0.1$; day 21: $P=0.94$, $F_{1,44}<0.1$). No other terms or interactions were significant for wing length (Table 1).

Haematocrit showed no significant age×brood or age×year interactions ($P>0.3$; Table 1). First brood chicks had 14.9% higher haematocrit than second broods on day 17 (42.9 ± 5.1 versus $36.5\pm 5.0\%$), and 10.5% higher than second broods on day 21 (46.6 ± 4.5 versus $41.7\pm 5.0\%$; Table 1). Brood also interacted with year ($P<0.01$, $F_{1,171}=8.8$) and sex ($P=0.03$, $F_{1,383}=4.6$): first broods had higher haematocrit in 2014 ($P<0.05$, $F_{1,113}=5.3$, controlling for sex and interactions), but second broods were equally low across years ($P=0.32$, $F_{1,12}=1.1$). Sex was not significant once age classes were separated between first broods (day 17: $P=0.67$, $F_{1,61}=0.2$; day 21: $P=0.86$, $F_{1,66}>0.1$, controlling for year and year×sex) and second broods (day 17: $P=0.34$, $F_{1,16}=1.0$; day 21: $P=0.13$, $F_{1,14}=2.5$).

For haemoglobin concentration there was a significant brood×age interaction in the model: in chicks from first broods haemoglobin increased by +6.9% between days 17 and 21, but at a much higher rate (+25.4%) from a lower initial value in second broods (Fig. 1). First broods had higher day 17 (12.7 ± 2.9 g dl⁻¹ versus second brood 10.1 ± 2.0 g dl⁻¹) and day 21 (13.3 ± 1.9 g dl⁻¹ versus second brood 12.1 ± 2.0 g dl⁻¹) haemoglobin concentrations (Table 1; Fig. 1) Year and sex were both unrelated to haemoglobin as main effects and in all possible combinations of interactions ($P>0.05$).

Oxidative stress and development

Reactive oxygen metabolites (d-ROMs) at day 21 were higher in second broods and in 2013: d-ROMs averaged 2.23 ± 0.83 mg

Table 1. Results from linear mixed effects models of physiological and somatic traits

Metric		Haematocrit	Haemoglobin	Body mass	Wing length
Age	<i>P</i> value	<0.01	<0.01	<0.01	<0.01
	<i>F</i> statistic	51.0	14.8	34.0	444.3
Brood	<i>P</i> value	<0.01	<0.01	<0.01	0.23
	<i>F</i> statistic	176	24.3	8.6	1.4
Age×brood	<i>P</i> value	0.37	0.03	0.43	0.23
	<i>F</i> statistic	1.1	4.8	0.6	1.4
Year	<i>P</i> value	0.71	0.22	<0.01	0.81
	<i>F</i> statistic	0.1	1.5	19.7	<0.1
Age×year	<i>P</i> value	0.89	0.93	0.36	0.60
	<i>F</i> statistic	0.2	<0.1	0.8	0.3
Sex	<i>P</i> value	<0.01	0.32	0.33	0.03
	<i>F</i> statistic	18.3	1.0	0.9	4.7
Age×sex	<i>P</i> value	0.99	0.46	0.72	0.37
	<i>F</i> statistic	<0.1	0.5	0.1	0.8

Physiological traits: mass and wing length; somatic traits: haematocrit and haemoglobin concentration. Age represents whether the measurement was taken 17 or 21 days after hatching, and interactions with age represent an effect on the developmental change (or slope) between day 17 and day 21. Models included age, brood, year and sex as independent main effects and all possible interactions, and parent ID as a random effect. Interactions between age, year and sex are only reported in text where significant.

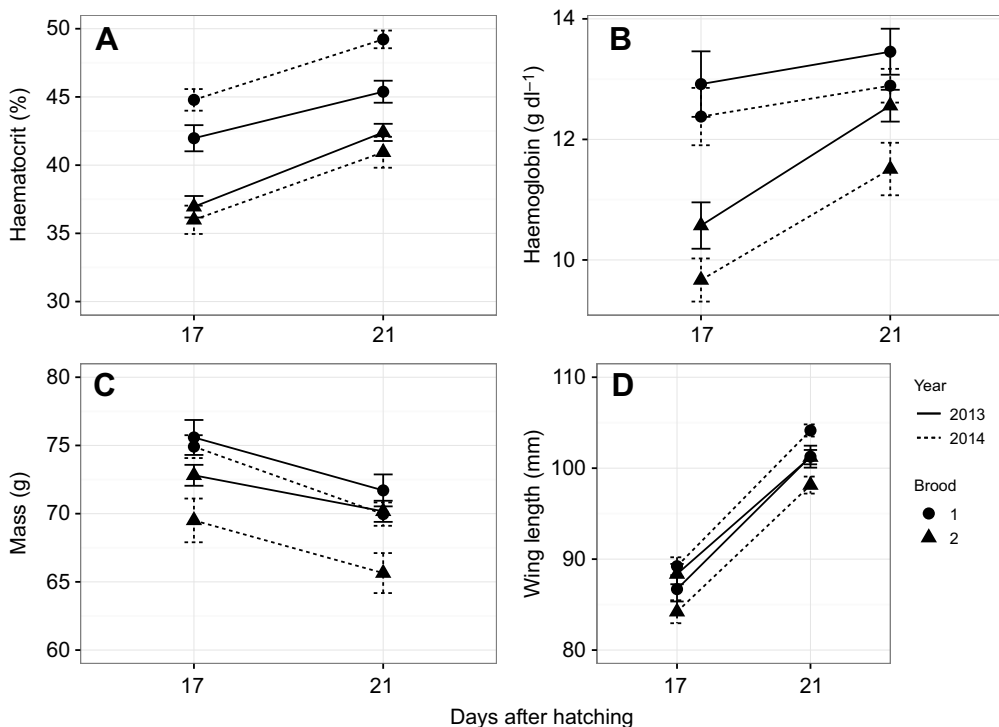


Fig. 1. Natural variation in change of physiological and somatic traits in the European starling (*Sturnus vulgaris*) just prior to and at fledging across high- and low-quality years and broods. (A,B) Physiological traits: mass and wing length. (C,D) Somatic traits: haematocrit and haemoglobin concentration. Error bars represent 95% confidence intervals. See Table 1 for significance of each comparison.

H_2O_2 dl^{-1} (first broods) and 2.59 ± 0.97 $\text{mg H}_2\text{O}_2$ dl^{-1} (second broods) in 2013, compared with 1.30 ± 0.44 $\text{mg H}_2\text{O}_2$ dl^{-1} (first broods) in 2014 and 1.26 ± 0.37 $\text{mg H}_2\text{O}_2$ dl^{-1} (second broods; Fig. 2). Anti-oxidant capacity (OXY) was also higher in 2013 than 2014; however, the ratio of d-ROMs to OXY was still higher overall in 2013 than 2014 (Table 2). This ratio also showed a significant relationship to brood, with second broods showing 18% higher oxidative stress values than first broods. Sex was unrelated to all oxidative stress metrics, as were all interactions between year, brood and sex ($P > 0.05$), with the exception of anti-oxidants and sex \times year: 2014 females (1.99 ± 0.54 $\mu\text{mol HOCl ml}^{-1}$) having higher anti-oxidant levels than males (1.76 ± 0.43 $\mu\text{mol HOCl ml}^{-1}$). All metrics of oxidative stress (OXY, d-ROMs, ratio of d-ROMs to OXY) were independent of individual variation in the per cent change in somatic or physiological traits ($P > 0.05$; Fig. 3; Fig. S1). We analysed these data both as per cent change from the day 17 value and as the raw change value and with no change to the null result ($P > 0.05$).

Supplemental feeding experiment

Supplemental feeding had a significant, and immediate, positive effect on mass gain during the nestling period (Fig. 4). Seven days after hatching, and 3 days after receiving supplementary meals, chicks in the day 4 treatment group were significantly heavier than

controls ($P < 0.01$, $F_{1,54} = 20.9$, controlling for brood, sex and interactions). Chicks in the day 4 treatment group maintained higher mass at days 10 and 16 (day 10: $P < 0.01$, $F_{1,54} = 14.7$; day 16: $P < 0.01$, $F_{1,54} = 10.0$ controlling for brood, sex and interactions), but this difference was not found on fledging date (day 21: $P = 0.45$, $F_{1,55} = 0.6$, controlling for brood, sex and interactions; Fig. 4). In day 16 and day 21 mass models, brood was a significant main effect ($P < 0.05$), but there was no significant effect of treatment \times brood interaction in any of the models (brood \times treatment: $P > 0.52$). Chicks that experienced supplemental feeding starting on day 10 (first brood chicks only) had increased mass after 4 days of feeding compared with controls ($P = 0.04$, $F_{1,28} = 4.6$, controlling for sex), but no difference was found later in the nestling period (day 17: $P = 0.63$, $F_{1,27} = 0.2$) or after the period of mass loss on day 21 (day 21: $P = 0.52$, $F_{1,27} = 0.4$; Fig. 4).

First brood chicks fed beginning on day 4 were not significantly different from chicks fed beginning on day 10 (all first broods) for change in mass, wing, haematocrit or haemoglobin from day 17 to day 21 ($P > 0.40$, controlling for sex, sex \times treatment, and parent ID as a random factor) and the same was true for corresponding control groups ($P > 0.16$). Therefore we combined day 4 and day 10 groups for analyses on these metrics. Supplemental feeding had no effect on the developmental trajectory (% change) for any somatic or

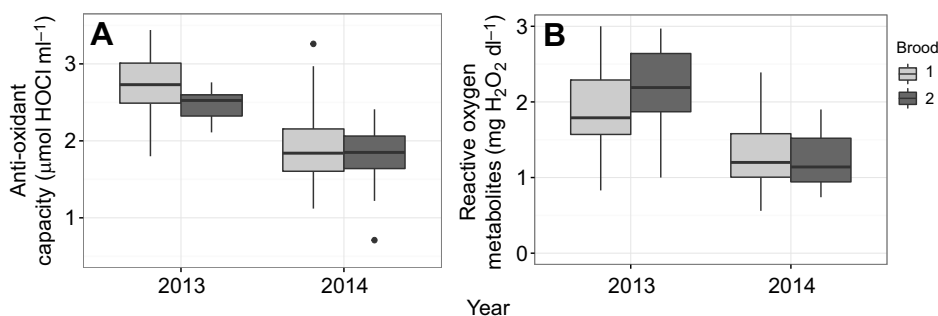


Fig. 2. Anti-oxidant capacity and reactive oxygen metabolites in a low productivity (2013) and high productivity (2014) year, and across broods of high (brood 1) and low (brood 2) productivity. (A) Anti-oxidant capacity; (B) reactive oxygen metabolites. Error bars show first and third quartiles; see Table 2 for significance.

Table 2. Results from linear mixed effects model of oxidative stress metrics and ecological context (year, brood) and sex, controlling for parent ID as a random factor

Metric	d-ROMs		OXY		OXY/d-ROMs	
	<i>P</i> value	<i>F</i> statistic	<i>P</i> value	<i>F</i> statistic	<i>P</i> value	<i>F</i> statistic
Year	<0.01	13.5	<0.01	32.5	<0.05	4.6
Brood	<0.01	7.5	0.15	2.1	<0.01	7.2
Sex	0.54	0.3	0.99	<0.1	0.86	<0.1
Year×brood	0.11	2.6	0.53	0.4	0.12	2.5
Year×sex	0.53	0.4	<0.05	7.1	0.71	0.1
Brood×sex	0.67	0.2	0.91	<0.1	0.62	0.3
Year×brood×sex	0.17	2.0	0.23	1.5	0.20	1.6

OXY, anti-oxidants; d-ROMs, reactive oxygen metabolites. Model included all metrics independently and as interactions.

physiological traits from day 17 to day 21 (all $P>0.16$, controlling for brood, sex and interactions, and parent ID as a random factor).

There was no significant difference in d-ROMs, OXY or the ratio of d-ROMs to OXY at day 21 comparing day 4- and day 10-fed chicks ($P>0.08$ in all cases), or among control chicks in the nests of these different food treatments ($P>0.17$ in all cases). Therefore we combined day 4 and day 10 chicks for analyses of oxidative state. Supplemental feeding had no effect on d-ROMs or the ratio of d-ROMs to OXY ($P>0.05$, controlling for brood, sex and interaction terms). However, there was an effect of supplemental feeding for OXY ($P=0.02$, $F_{1,73}=6.0$) and an interaction with brood ($P<0.01$, $F_{1,75}=7.3$). First brood chicks had no difference between treatment and control groups ($P=0.10$, $F_{1,36}=2.8$), but food-treated second brood chicks had higher anti-oxidant capacity (1.98 ± 0.35 $\mu\text{mol HOCl ml}^{-1}$) than control chicks (1.75 ± 0.31 $\mu\text{mol HOCl ml}^{-1}$; $P<0.05$, $F_{1,30}=4.3$).

DISCUSSION

Here we tested, both correlationally and experimentally, whether variation in developmental trajectories prior to fledging varied for somatic versus physiological traits, and in relation to ecological context or food resources. Not surprisingly, we observed changes in somatic (mass, wing length) and physiological (haemoglobin

concentration, haematocrit) traits just prior to fledging in chicks across years, broods and experimental treatments. Wing length, haematocrit and haemoglobin all increased prior to fledging whereas body mass decreased (-5.2%), and the developmental trajectory (slope) for body mass, wing length and haematocrit was unrelated to ecological context, potentially consistent with Ricklefs and Starck's (1998) third level of physiological constraint. In contrast, the developmental trajectories for haemoglobin did vary with one aspect of ecological context (brood number): chicks in second broods had lower haemoglobin at day 17 (more physiologically immature) but a greater rate of increase between days 17 and 21, although again this pattern was consistent between years. Despite the relative inflexibility of developmental trajectories for most somatic/physiological traits, we did observe variation in a potential physiological cost of development: chicks in both the lower productivity year (2013) and lower quality brood (second) showed higher oxidative stress at fledging. This suggests that although chicks appear to have 'hardwired' developmental trajectories, they might pay a greater cost for maintaining this rate of development in low-quality ecological conditions. Results from our experimental manipulation were consistent with our correlational data: although supplemental feeding increased body mass up to day 17 this did not affect developmental trajectories just

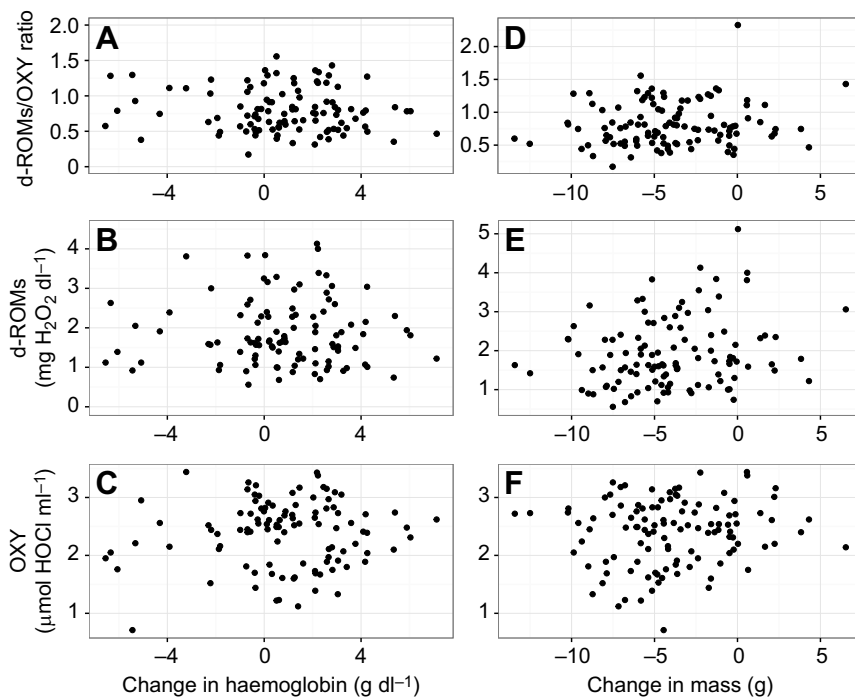


Fig. 3. Anti-oxidant capacity, reactive oxygen metabolites and ratio of reactive oxygen metabolites to anti-oxidant capacity and change in haemoglobin concentration and change in mass from day 17 to day 21. (A–C) Change in haemoglobin concentration; (D–F) change in mass. Each data point represents one individual chick; no relationships were significant.

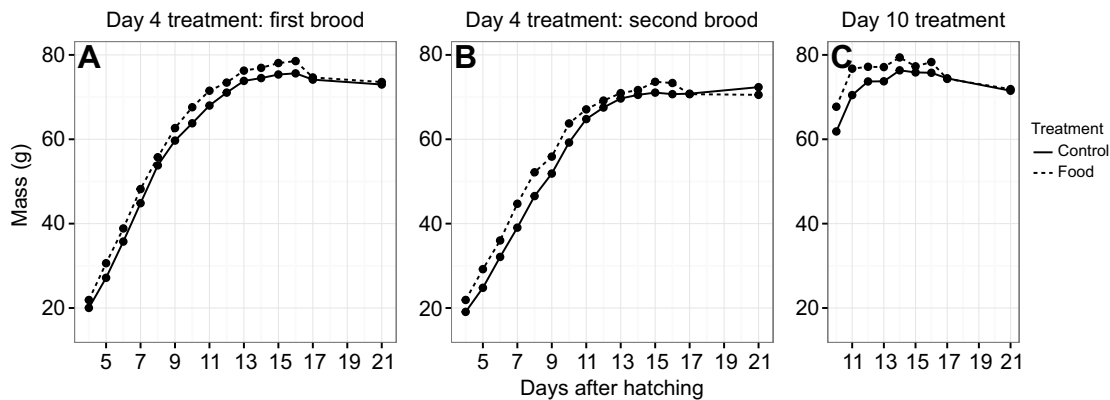


Fig. 4. Mass change throughout the nestling period in food treatment and control chicks from first and second broods. (A) First brood (20 fed chicks, 18 control chicks); (B) second brood (22 fed chicks, 23 control chicks). Chicks were not measured on days 18, 19 and 20. (C) Fed ($n=23$) and control ($n=10$) chicks began treatment on day 10. This day 10 treatment was only conducted in first broods. Chicks from both control and treatment groups were from the same nests in first broods (12 nests), second broods (17 nests) and day 10 treatments (eight nests). Chicks were weighed at the end of each day (after their supplemental meals), including on day 4.

prior to fledging, or developmental maturity at fledging, for somatic or physiological traits.

In European starlings, wing length, haematocrit and haemoglobin all showed a significant increase towards adult values whereas body mass decreased prior to fledging (see Cornell et al., 2016a,b for adult values in this species). In other species, chicks have been shown to maintain or even decrease haematocrit and haemoglobin concentration just prior to fledging (Simmons and Lill, 2006). However, few have studied development during this period in a ground foraging songbird. Adaptive pre-fledging mass loss has been documented previously, although mostly in seabirds and aerial insectivores (Ricklefs, 1968; Martins, 1997; Morbey et al., 1999; Mauck and Ricklefs, 2005; Wright et al., 2006). Flight is surely critical to the survival of many birds, including ground foraging starlings, although perhaps more important for birds that forage on the wing. Some suggest that this decrease in mass optimizes wing loading (ratio of wing area to body mass), especially important for species that use flight for foraging. This hypothesis has been shown to be true experimentally for swifts (Wright et al., 2006), a species dependent on foraging flights. As one would expect, the mass recession we show in ground foraging starlings (-5%) is less than that of seabirds (-35% of peak chick mass, *Oceanodroma castro*; Allan, 1962) and swallows (-22% , *Tachycineta thalassina*; Edson, 1943), which depend on foraging flights. It is difficult to draw comparative conclusions from only a few representative species, and there may be other influential factors, such as variation in post-fledging parental care. Because the mass loss in starlings was not proportional to increases of other traits during this period, we have no evidence to suggest that starlings lose mass as a by-product of development of other traits.

Developmental changes in the traits we measured prior to fledging were largely unrelated to ecological context, as indicated by year differences in productivity and brood number (typically reflecting a seasonal decline in rearing environment). We predicted that the slope of development, or interactions with age in our model, might be affected by year quality (Winkler et al., 2013; Mainwaring and Hartley, 2016; Pérez et al., 2016) and brood quality (second broods have lower survival: Verboven and Visser, 1998; Naef-Daenzer et al., 2001; Lambrechts et al., 2008; Gruebler and Naef-Daenzer, 2010). However, haemoglobin concentration, a trait linked to both pre- and post-fledging survival (Nadolski et al., 2006; Bowers et al., 2014; Kaliński et al., 2015), was the only trait for which brood affected

slope of development. In this relationship, second brood birds showed a significantly higher increase in haemoglobin concentration over the 4 days prior to fledging ($+25.4\%$), compared with first broods ($+6.9\%$). Despite the higher increase, second brood chicks still fledged with lower haemoglobin concentrations than first broods (Cornell et al., 2016a,b), suggesting that they have reduced development prior to our first day 17 measurement. Wing length, body mass and haematocrit developmental changes had no relationship to year or brood, suggesting that the ecological context does not influence developmental trajectory of these traits just prior to fledging. We also measured the presence of haematological ectoparasites in the nest in 2013, as an additional aspect of ecological context. However, we found almost no ectoparasites (4/68 nests infested) and therefore no significant effect of ectoparasite on our population. Given the considerable risk of mortality (55–80%) immediately post-fledging (Adams et al., 2001; Anders et al., 2002; Kershner et al., 2004; Moore et al., 2010), it is possible that hardwired developmental patterns just prior to this high-risk period may be adaptive to ensure fledging at a minimum maturity level. Our data therefore support Ricklef's hypothesis that developmental trajectory may be determined, at least somewhat, by 'internal physiological constraints' (Ricklefs, 1968, 1973).

Reactive oxygen metabolite concentration was highest in the low productivity year and brood, suggesting higher oxidative damage (Murphy et al., 2011). Although anti-oxidant capacity was also higher in the low productivity year, this was not enough to compensate for the high levels of reactive oxygen metabolites, as the ratio of reactive oxygen metabolites to anti-oxidant capacity was still higher overall in the low productivity year. Consistent with other studies, we found no relationship between sex and oxidative stress (Alonso-Alvarez et al., 2006; Costantini et al., 2006; Noguera et al., 2011). The seasonal effects are in contrast to previous findings in Eurasian kestrel nestlings (*Falco tinnunculus*), which found no relationship between hatching date and oxidative damage (Costantini et al., 2006), and in European shag (*Phalacrocorax aristotelis*) chicks, which had no correlation between hatching date and lipid peroxidation (Noguera et al., 2011). Our results suggest that although chicks maintain a similar developmental trajectory despite varying ecological context, there may be oxidative costs for maintaining this potentially hardwired development in lower quality environments. Other studies have already demonstrated that rapid growth can have costs in the form of oxidative stress (reviewed in

Metcalfé and Alonso-Alvarez, 2010). It is possible that in low-quality environments, our chicks incur the additional long-term cost of oxidative stress (Hulbert et al., 2007) by maintaining the same developmental trajectory as a high quality year or brood. Because of the high predation rate at fledging, this long-term oxidative cost may be adaptive over the potential short-term cost of fledging at immaturity in a high mortality period, despite the relationship between higher oxidative damage and lower long-term recruitment rate (Noguera et al., 2011). At the individual level, we found no relationship to the slope of developmental change and reactive oxygen metabolites, anti-oxidant capacity, or ratio of these measures, when controlling for significant year and brood effects. This may be due to other factors, such as genetics or individual optimization, regulating the individual level of cost that we did not measure. Similarly Costantini et al. (2006) found that Eurasian kestrel nestlings appeared to have alternative mechanisms for optimizing growth: males and females with very different sex-based growth trajectories had similar oxidative stress.

Our feeding experiment was an attempt to manipulate the ecological context for development directly by providing extra resources to half of the chicks in each nest. It is unlikely that parental provisioning compensated for the within-nest variation in treatment groups given that chicks initially did show higher masses as a result of supplemental feeding, and the evidence that hunger of chicks does not necessarily correlate with parental feeding in a straightforward way (Ostreiher, 1997; Krebs, 2001; Roulin, 2001). However, if parents did feed control chicks more than treatment chicks, then the significant mass differences we observed should be underestimates. Chicks fed since day 4 initially responded with an increase in mass from day 7 to day 17; however, during the period of mass recession prior to fledging, chicks receded to masses equivalent of control chicks. This is consistent with other studies which have found that food quantity may not be directly related to growth (Harris, 1978; Hodum and Weathers, 2003). It is worth noting that the food treatment was not administered during the mass recession period from day 17 to day 21, nor were treated or control chicks weighed daily during this period in order to prevent accidental early fledging. In contrast to the chicks fed beginning on day 4, day 10-treated chicks did not have sustained mass gains, and were equal to controls in mass by day 17 even though they were fed until and on this date. In addition, there were no effects of food treatment on chicks' wing length, haematocrit or haemoglobin concentration on day 17, day 21, or developmental change between these days. Thus our experimental evidence is similar to data on natural variation: ecological context and increased food did not affect maturity at fledging or developmental change just prior to fledging, suggesting that developmental trajectories may be hardwired for the traits we measured. However, food-treated birds did show 12% higher anti-oxidant capacity in the lower quality brood (second), a trait that has been linked to post-fledging survival (Noguera et al., 2011). This suggests that food-treated birds may have had reduced oxidative costs of maintaining a potentially hardwired growth pattern. This is consistent with other experimental studies demonstrating that nutrition affects anti-oxidant levels in developing chicks (Blount et al., 2003). Collectively, our natural and experimental data show that developmental trajectories of the traits we measured may be hardwired as there was little variation in trajectory of development prior to fledging based on ecological context or treatment group. However, we do present evidence of a potential cost for maintaining these developmental trajectories in lower quality environments or control groups in the form of higher oxidative stress.

Acknowledgements

Some work from this article's Introduction, Methods, Results and Discussion is reproduced from Allison Cornell's PhD thesis (Simon Fraser University, 2017). We would like to thank undergraduate assistants Megan Rogers, Laramie Ferguson, Kate Gibson, James Hou and Joyce Ma as well as Dr Melinda Fowler and Mitchell Serota for their pivotal role in collecting field data. We would also like to thank the Davis family for generously allowing us to use their farm as our field site.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.C., T.D.W.; Methodology: A.C.; Formal analysis: A.C.; Investigation: A.C.; Data curation: A.C.; Writing - original draft: A.C.; Writing - review & editing: A.C., T.D.W.; Visualization: A.C.; Supervision: T.D.W.; Project administration: T.D.W.; Funding acquisition: T.D.W.

Funding

This work was funded by a Natural Sciences and Engineering Council of Canada Discovery and Accelerator Grant to T.D.W. (grant numbers 155395-2012-RGPIN and RGPAS/429387-2012).

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.162248.supplemental>

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