The Journal of Experimental Biology 203, 3513–3520 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB3125

EFFECT OF BROOD SIZE MANIPULATION ON OFFSPRING PHYSIOLOGY: AN EXPERIMENT WITH PASSERINE BIRDS

GARY P. BURNESS[1,*], GRANT B. McCLELLAND[1,‡], SHARILYNN L. WARDROP[2] AND PETER W. HOCHACHKA[1]

[1]*Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4* and [2]*Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6*

*Present address: Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90095-1751, USA (e-mail: burness@zoology.ubc.ca)

‡Present address: University of California at Berkeley, Department of Integrative Biology, Berkeley, CA 94720-3140, USA

Accepted 21 August; published on WWW 24 October 2000

Summary

The environment experienced during ontogeny has a significant impact on the physiological condition of offspring. This, in turn, forecasts survival probabilities and future reproductive potential. Despite the prominent role that the concept of condition plays in evolutionary studies, the physiological and biochemical characters that define it remain relatively unexplored. In this study, we quantified the impact of brood size manipulations on the physiology and biochemistry of nestling tree swallows (*Tachycineta bicolor*) shortly before they fledged. Over two breeding seasons, we either increased or decreased the number of individuals in a brood by a single nestling. Every 2–4 days, we determined the resting rate of oxygen consumption ($\dot{V}_{O_2}$) of individuals in each brood. Growth was followed until 16 days of age, at which time, to look for potential trade-offs in energy allocation, we measured total lipid mass, skeletal muscle and organ mass, indices of blood oxygen-carrying capacity and the activities of key metabolic enzymes in various tissues. Surprisingly, there was a minimal response of most characters to brood manipulation, suggesting that physiological and biochemical development is relatively invariant except perhaps under extreme conditions. Individuals reared in artificially enlarged broods, however, had a significantly lower body mass, body-size-adjusted $\dot{V}_{O_2}$, gizzard mass and total lipid mass. These individuals also had decreased activity of cardiac 3-hydroxyacyl CoA dehydrogenase, suggesting a decreased capacity for oxidation of fatty acids. How these characters affect survival or the future adult phenotype remains unknown.

Key words: environmental effect, plasticity, tree swallow, *Tachycineta bicolor*, metabolism, enzyme, brood size.

Introduction

The environment experienced during avian and mammalian ontogeny can have important morphological, behavioural and life history consequences. Individuals raised under poor conditions often exhibit smaller structural size, are lighter in mass at independence and have decreased over-winter survival and recruitment rates (e.g. Perrins, 1965; Boag, 1987; Richner, 1989; Dijkstra et al., 1990; Koskela, 1998). As adults, these individuals may breed in low-quality habitats (Verhulst et al., 1997), have reduced fecundity (e.g. smaller clutch sizes; Haywood and Perrins, 1992; Schluter and Gustafsson, 1993) or decreased attractiveness of secondary sexual characters (Gustafsson et al., 1995; de Kogel and Prijs, 1996).

Implicit in the above studies is the assumption that variation in the quality of the rearing environment has an effect on the 'physiological condition' of individuals reared in that environment. Differences in condition at fledging are then manifest through survival probabilities and variation in the adult phenotype (Perrins, 1965; Haywood and Perrins, 1992; Schluter and Gustafsson, 1993; de Kogel and Prijs, 1996). Despite the prominent role that the concept of condition plays in many evolutionary studies (e.g. McNamera and Houston, 1992), the physiological and biochemical characters that define it remain relatively unexplored.

A number of previous studies have linked intra-specific variation in body composition with measures of whole-animal performance (e.g. Garland, 1984; Røskaft et al., 1986; Chappell et al., 1999; Hammond et al., 2000). This led us to hypothesize that the size and metabolic activity of skeletal muscles and various internal organs may be indices of 'condition'. Although some of these indices have a genetic basis (e.g. Garland et al., 1990), they are probably susceptible to environmental variation.

We used the tree swallow (*Tachycineta bicolor*) to investigate whether the environment experienced during ontogeny would

affect the physiology and metabolism of nestlings shortly before fledging. We experimentally manipulated brood size and determined the resting rate of oxygen consumption ($\dot{V}_{O_2}$) of individuals reared in each brood. To look for potential trade-offs in energy allocation near fledging, total lipid mass and the masses of skeletal muscle and internal organs were measured. Since we hypothesized *a priori* that differences in condition might also be linked to differences in blood oxygen-carrying capacity, we measured blood haemoglobin concentration and haematocrit. Finally, the activities of the following key metabolic enzymes were measured in various tissues: (i) citrate synthase (an index of aerobic capacity), (ii) pyruvate kinase (an index of glycolytic capacity), (iii) 3-hydroxyacyl CoA dehydrogenase (an index of the capacity for fatty acid catabolism) and (iv) lactate dehydrogenase (an index of the capacity for anaerobic glycolysis).

Materials and methods

Study site and species

The field component of this study was performed in May–June 1996 and 1998 at the Creston Valley Wildlife Area, near Creston, British Columbia, Canada. Beginning in early May, we began checks of nest boxes for signs of breeding by tree swallows (*Tachycineta bicolor*). Females in this population lay up to eight eggs, with a modal clutch of six. Clutch completion is followed by 12–14 days of incubation. Nestlings hatch relatively synchronously (over 1–2 days), follow a sigmoidal growth curve and reach maximum mass at approximately day 12 (hatch day=day 1). This is followed by a weight recession that continues until fledging at 18–22 days of age (for a review, see Robertson et al., 1992).

Manipulation of nestling environment

In both years of study, manipulations consisted of either increasing or decreasing the number of nestlings in a brood. One nestling was either added to or removed from a nest on day 4 (1996) or day 6 (1998). All nestlings were banded, and the growth of members of the brood was followed until day 16. We did not use a control group because we were interested only in demonstrating an effect of manipulation and not in predicting the directionality of the response (i.e. an increase *versus* a decrease in a given character). In 1996, we used only nests in which females had laid six eggs; because of a shortage of suitable nests in 1998, we used both five- and six-egg nests. It is not known whether differences in protocol between years affected our measurements; consequently, the term ‘year’ was included in all statistical analyses.

Morphometrics

All nestlings in experimental broods were weighed (±0.5 g) on either day 4 (1996) or day 6 (1998) and then again on days 8, 12 and 16. Day 16 is the last day that nestling can be handled without the risk of premature fledging (De Steven, 1980). At day 16, the nestling with the mass closest to the average for a given brood had the following additional measurements taken: tarsus length, total body length, middle toe and keel lengths, bill length, bill depth and bill width. In addition, the length of the ninth primary feather (plucked) was measured because its length at 16 days of age correlates with age of nest departure (De Steven, 1980). To minimize inter-observer variability, the same investigators performed all measurements in both years for a given character.

Resting rate of oxygen consumption

In 1998, on days 6, 8, 12 and 16, the nestling of average mass from each brood was transported to the field laboratory (different nestlings each day). Resting rate of oxygen consumption ($\dot{V}_{O_2}$) was measured using a flow-through respirometry system (Sable Systems TR-3, Henderson, NV, USA). Within approximately 30 min of removal from the nest, nestlings were placed in a black Plexiglas metabolic chamber with a volume of either 500 ml or 1000 ml depending on the size/age of the nestling. The air inlet and outlet of the metabolic chamber consisted of brass tubes, extending from the top to the bottom of the chamber and perforated along their lengths to maximize mixing of air within the chamber. The chamber was placed in a temperature-controlled cabinet. The temperature inside the chamber was maintained at 32.1–33.0 °C and was continuously monitored using a thermocouple placed in the air outlet of the metabolic chamber.

Water- and carbon-dioxide-free air was drawn through the metabolic chamber at 200–500 ml min^{-1} using a combination pump/mass-flow meter (Sable Systems TR-SS1). A sub-sample of out-flowing air was drawn through an Amatek S-3A oxygen analyzer at 150–200 ml min^{-1} after being dried with magnesium perchlorate, $Mg(ClO_4)_2$. Measurements were taken for 60 min, and the lowest $\dot{V}_{O_2}$ recorded over a continuous 5 min period during the last 30 min was used in calculations of resting $\dot{V}_{O_2}$ using the equations of Withers (1977). The oxygen analyzer was precise to ±0.001 %, and the system was accurate to ±1.0 %. $\dot{V}_{O_2}$ was corrected to STPD.

Frequently, two nestlings were brought to the laboraory simultaneously. In these cases, to minimize metabolic variation due to differences in the degree of post-absorptiveness, one individual was placed in a chamber for 60 min (as above), and the other was fed approximately 0.4 g of moistened cat food (Vineland, Abottsford, British Columbia, Canada). The fed individual was then placed in a duplicate metabolic chamber, and both were then placed in the temperature-controlled cabinet. For the fasted individual, the time elapsed between last possible feeding and the first measurement of $\dot{V}_{O_2}$ was 60 min. For the individual fed in the laboratory, measurement was a minimum 90 min post-feeding. There was no systematic bias between the two treatments. Following metabolic trials, birds were removed from the chamber, reweighed and, if 6, 8 or 12 days old, re-fed and returned to their nest. Nestlings that were 16 days old were retained for additional measurements (below).

Blood variables

A 100–200 µl blood sample was collected from each 16-day-

old nestling into heparinized microcapillary tubes. To determine haematocrit (Hct), microcapillary tubes were centrifuged at maximum speed for 10 min using an Adams micro-haematocrit bench-top centrifuge. Haemoglobin (Hb) concentration was determined in the field using a portable HemoCue B-Hemoglobin photometer (Ängelholm, Sweden). The number of replicates for each character was determined by the size of the blood sample and ranged from one to three (which were averaged).

Carcass analyses

Day 16 nestlings were killed immediately after blood sampling (following the guidelines of the Canadian Committee on Animal Care). Samples (approximately 150 mg) of the right pectoralis major muscle and liver were removed from each bird (within 1–2 min of death) and immediately frozen in a dry shipper charged with liquid N_2. These samples were later transferred to liquid N_2 for storage for 3 months. The pectoralis and supracoracoideus were subsequently removed, followed by the heart, liver, gizzard, small intestine and kidney. All tissues except the gizzard were stored in air-tight cryovials, frozen in the dry shipper and, within 1 month of collection, were transferred to a −80 °C freezer. The remainder of each carcass (including the gizzard) was double-bagged and frozen at −20 °C.

Carcasses were weighed (to ±0.0001 g) upon removal from the freezer and plucked of all feathers. All muscles on the tibiotarsus and femur were then removed from one side of the bird, rinsed with 0.9 % NaCl, blotted dry and weighed. To calculate total leg muscle mass, values were multiplied by 2.

Wet masses were determined for all additional organs and tissues (±0.0001 g). The small intestine was cut into three sections of equal length. The gizzard and each section of the small intestine were then cut longitudinally and the contents rinsed out with 0.9 % NaCl. Each tissue was then blotted dry and reweighed.

In preparation for fat extraction, all organs and tissues (with the exception of the heart, liver and kidney) were freeze-dried to constant mass. Carcasses were dried to constant mass in an oven at 70 °C. All samples were then fat-extracted for 7 h in a Soxhlet apparatus containing petroleum ether as the solvent (Dobush et al., 1985). Following extraction, samples were placed in a fume hood to evaporate any remaining solvent, oven-dried overnight and then reweighed. The difference between the pre-extraction and post-extraction mass represents the lipid mass.

Enzyme assays

Subsamples (approximately 150 mg) of the pectoralis major and liver and the ventricles of the heart were weighed frozen (to ±0.0001 g) and added to nine volumes of homogenization buffer [20 mmol l^{-1} Na_2HPO_4, 0.2 % bovine serum albumin, BSA (defatted), 5 mmol l^{-1} β-mercaptoethanol, 0.5 mmol l^{-1} EDTA, 100 μg ml^{-1} aprotinin, 50 % v/v glycerol, pH 7.4 at 21 °C; Mommsen and Hochachka, 1994] at 0 °C. Each sample was minced on ice for 1 min using scissors, followed by homogenization using a hand-held Tissue Tearor (three 10 s bursts separated by 30 s breaks). Samples were further homogenized for 3 min using a Lurex ground-glass-on-glass homogenizer, and then sonicated for three 10 s bursts, separated by 30 s breaks, using a Kontes Micro-ultrasonic cell disrupter. Homogenates were stored at −80 °C until they were assayed (maximum 1 month). This homogenization medium allows samples to be frozen for extended periods with no loss of enzyme activity (Mommsen and Hochachka, 1994).

As an index of capacity for flux through various metabolic pathways, we measured the maximum catalytic activity of key metabolic enzymes under optimal conditions. All assays were performed on a 96-well Thermomax microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). In all assays, uncentrifuged homogenates were used to avoid potential loss in the pellet. Each reaction was replicated in five wells. The activities of the highest and lowest wells were omitted, and the remaining values were averaged. Preliminary experiments confirmed that levels of all substrates and cofactors were saturating but not inhibitory. Initially, control wells (containing no substrate) were run simultaneously with all reactions. The control rates for pyruvate kinase and lactate dehydrogenase represented less than 2 % of total activity and were omitted in subsequent assays. Control wells were included for all 3-hydroxyacyl CoA dehydrogenase and citrate synthase assays. With the exception of citrate synthase, all assays were performed at pH 7.0 with detection at 340 nm. Citrate synthase was assayed at pH 8.0 with detection at 412 nm. All reactions were carried out at 40 °C. Activities are expressed as international units (μmoles of substrate converted to product per minute) per gram wet mass of tissue.

Assays were performed as follows. Citrate synthase (EC4.1.3.7; CS): 50 mmol l^{-1} Tris buffer, 0.05 % Triton X-100, 0.2 mmol l^{-1} 5,5′dithiobis(2-nitrobenzoic acid) (DTNB), 0.12 mmol l^{-1} acetyl CoA, 0.5 mmol l^{-1} oxaloacetate (omitted from the control well). 3-Hydroxyacyl CoA dehydrogenase (EC1.1.1.35; HOAD): 50 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN, acetoacetyl CoA (0.1 mmol l^{-1} for the pectoralis and ventricle, 0.05 mmol l^{-1} for the liver; omitted from the control well). Pyruvate kinase (EC2.7.1.40; PK): 50 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN, 5.0 mmol l^{-1} ADP, 100 mmol l^{-1} KCl, 10 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} phosphoenolpyruvate (PEP), 10 μmol l^{-1} fructose 1,6-bisphosphate, excess lactate dehydrogenase (5 units ml^{-1}); assayed in the ventricles and pectoralis only. Lactate dehydrogenase (EC1.1.1.27; LDH): 20 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 2 mmol l^{-1} pyruvate, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN; assayed in the ventricles and pectoralis only.

Statistical analyses

Many physiological variables scale allometrically with body mass. In this study, the effect of brood manipulation on body mass was of interest. Consequently, rather than controlling for body mass, we instead controlled for structural size in most

analyses. To generate an index of size, we performed a principal component analysis (PCA) on the correlation matrix of seven external morphological variables (tarsus length, total body length, middle toe and keel lengths, bill length, bill depth and bill width). Loadings were positive for all variables and ranged from 0.24 to 0.47, with a corresponding Eigenvalue of 3.13. The first principal component (PC1) accounted for 44.7 % of the total original variance. We used the scores along PC1 as a measure of body size (e.g. Alisaukas and Ankney, 1987) with positive values representing individuals that were larger than average body size and negative values representing individuals that were smaller than average.

The effects of treatment (brood manipulation) and year on phenotypic variation of 16-day-old chicks were explored using a two-way analysis of covariance (ANCOVA) with body size included as a covariate. Initially, all interaction terms were included as additional covariates and if not significant were excluded. Further analyses were then performed including only the covariate and main effects (treatment and year). We used a liberal $P<0.15$ for inclusion of interaction terms; for main effects, significance was claimed at $P<0.05$. Unless noted otherwise, all means are least-squares means ± 1 S.E.M. All analyses were performed using JMP statistical software (SAS Institute Inc.).

Results

Growth and metabolic rate of nestlings

To determine the impact of brood manipulation on nestling growth, we averaged the mass of all individuals within a brood. On the day of manipulation (day 4 or 6), there was no difference between treatments in the mass of the average nestling ($P>0.50$, Fig. 1A). By 12 days of age, however, nestlings in the reduced broods were significantly heavier than those in the enlarged broods ($F_{1,29}=7.963$, $P=0.009$); this difference was maintained at 16 days ($F_{1,29}=8.857$, $P=0.006$, Fig. 1A).

Body-mass-adjusted $\dot{V}_{O_2}$ at 6, 8 and 12 days of age did not differ between treatments ($P>0.10$, Fig. 1B). By 16 days of age, the effect of brood manipulation on mass-adjusted $\dot{V}_{O_2}$ approached significance ($F_{1,11}=3.548$, $P=0.086$). We re-analyzed the $\dot{V}_{O_2}$ of the 16-day-old nestlings with structural size (PC1, rather than mass) included as a covariate. Individuals in reduced broods had a 15 % greater body-size-adjusted $\dot{V}_{O_2}$ than those in the enlarged broods ($F_{1,11}=6.108$, $P=0.0310$).

Morphology and physiology of 16-day-old nestlings

Brood manipulation had no effect on the composite index of structural size (PC1, $P=0.500$) or on the length of the ninth primary feather ($P=0.681$) at 16 days of age. In 1998, nestlings were structurally larger ($F_{1,29}=64.517$, $P<0.001$) and had longer primaries ($F_{1,29}=9.018$, $P=0.006$) than in 1996, but were no heavier ($P=0.484$).

To explore the basis of body mass differences resulting from brood manipulation (see above), we measured total lipid mass.

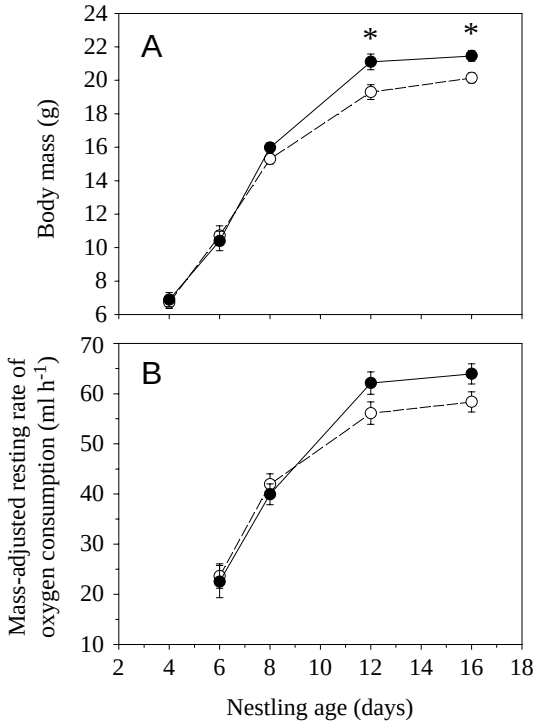

Fig. 1. (A) Body mass and (B) body-mass-adjusted resting rate of oxygen consumption of tree swallow nestlings as a function of age and treatment. Enlarged broods (○), reduced broods (●). Sample sizes (enlarged, reduced): A, day 4 (9, 6), day 6 (8, 8), days 8, 12 and 16 (17, 15); B, day 6 (6, 4), day 8, (8, 8), day 12 (8, 8), day 16 (7, 7). Values are least-squares means ±1 S.E.M. Asterisks indicate that treatments differed significantly from each other (ANOVA; *$P<0.01$).

PC1 was included as a covariate, with year and treatment as main effects. Nestlings from reduced broods had a 19 % greater lipid mass at day 16 than those from enlarged broods ($F_{1,28}=5.623$, $P=0.025$, Fig. 2). There was no difference between years ($P=0.486$).

After controlling for structural size, individuals in the reduced treatment had heavier organs than those in the enlarged treatment; however, only the mass of the gizzard showed a significant difference ($P<0.05$, Table 1). There was no effect of year on the wet mass of any organ except the intestine ($P<0.01$).

There was no effect of brood manipulation on the wet masses of either the pectoral or leg muscles ($P>0.05$, Table 1). The water content of a muscle (total water/lipid-free wet mass of tissue) decreases with chronological age (Konarzewski, 1988) and is a useful index of muscle maturation (Ricklefs and Webb, 1985). There was no significant effect of treatment on water fraction of either the pectoral or leg muscles ($P>0.10$, Table 1), suggesting that at 16 days of age individuals from the two treatments were of similar degrees of functional maturity. Significant year effects (Table 1) may be due to desiccation in the freezer; consequently, we assign them no particular functional significance.

As an index of blood oxygen-carrying capacity, we

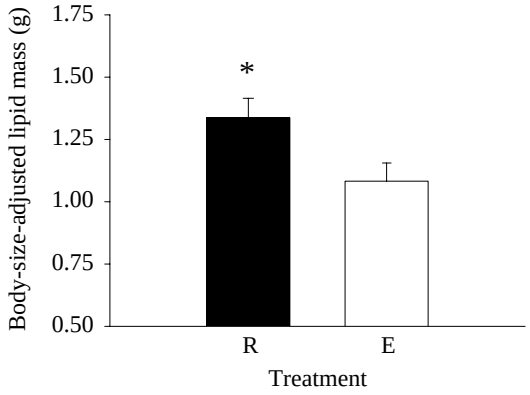

Fig. 2. Lipid mass of 16-day-old tree swallow nestlings, adjusted for body size (PC1; see text), as a function of brood manipulation. Reduced broods (R; filled column), enlarged broods (E; open column). Values are least-squares means +1 S.E.M. Reduced, N=15; enlarged, N=17. An asterisk indicates that treatments differed significantly from each other (ANCOVA; *P<0.05).

measured the Hct and Hb content of the blood. The average Hct was 41.7±4.50 % (N=32) and ranged from 27.3 to 51.0 %; the average Hb concentration was 13.5±2.06 g dl^{-1} (N=31) (means ± S.D.) and ranged from 6.7 to 16.3 g dl^{-1}. There were no significant effects of treatment or year on either character (P>0.25).

Enzyme activities

There was little effect of brood manipulation on maximum enzyme activities. Individuals from reduced broods had significantly higher HOAD activity in the heart (P<0.01, Table 2), suggesting an increased capacity for fatty acid oxidation. No other enzyme showed a significant difference between treatments.

Table 1. *Effect of brood manipulation on organ mass, muscle mass and muscle water fraction of 16-day-old tree swallows*

	Treatment		*P*	
	Enlarged brood	Reduced brood	Treatment	Year
Heart (g)	0.25±0.008	0.26±0.008	0.476	0.498
Liver (g)	0.90±0.030	0.98±0.032	0.074	0.990
Kidney (g)	0.25±0.011	0.27±0.012	0.149	0.927
Gizzard (g)	0.57±0.017	0.62±0.018	0.043	0.472
Intestine (g)	0.75±0.028	0.83±0.030	0.079	0.006
Pectoralis (g)	1.98±0.065	2.15±0.069	0.092	0.315
Water (g/g)	0.78±0.003	0.78±0.003	0.907	0.007
Leg (g)	0.66±0.020	0.62±0.021	0.181	0.946
Water (g/g)	0.78±0.006	0.77±0.007	0.154	<0.001

Values for organ and muscle masses are least-squares means ± 1 S.E.M., with P values from a two-way ANCOVA with body size as a covariate (PC1; see text).

Values for water fraction are least-squares means ± 1 S.E.M., with P values from a two-way ANOVA.

All interaction terms were non-significant (P>0.15).

Enlarged brood, N=17; reduced brood, N=15.

Table 2. *Maximum enzyme activities from tissues of 16-day-old tree swallows*

	Treatment		Statistic	
	Enlarged brood	Reduced brood	*F*	*P*
Heart				
CS	132.9±11.6	131.4±11.6	0.008	0.930
PK	146.8±13.6	173.9±13.6	1.937	0.187
LDH	61.5±5.4	74.9±5.4	3.019	0.106
HOAD	14.3±1.1	19.1±1.1	9.502	0.009
Pectoralis				
CS	136.3±12.6	137.6±12.6	0.006	0.941
PK	405.7±20.8	387.1±20.8	0.394	0.541
LDH	406.7±24.6	447.0±24.6	1.304	0.274
HOAD	10.3±1.0	9.1±1.0	0.867	0.369
Liver				
CS	11.3±0.7	9.7±0.7	2.757	0.121
HOAD	36.1±1.3	35.5±1.4	1.209	0.293*

Values are least-squares means ± 1 S.E.M., with P values from a one-way ANCOVA with body size as a covariate (PC1; see text).

CS, citrate synthase; PK, pyruvate kinase; LDH, lactate dehydrogenase; HOAD, 3-hydroxyacyl CoA dehydrogenase.

*Significant body size × treatment interaction (P<0.15).

Enzyme activity is in units g^{-1} tissue (μmol of substrate converted to product per minute). N=8 for each treatment.

Discussion

The brood size of tree swallows was manipulated to determine whether the environment experienced during ontogeny would affect the physiology and biochemistry of nestlings shortly before they were to fledge. As skeletal characters frequently respond to environmental variation during development (Lindström, 1999, and see references within), a minimal response at the physiological and biochemical level was surprising. A lack of variation in basal measures suggests that physiological and biochemical development may be relatively invariant except, perhaps, under extreme conditions (e.g. Schew and Ricklefs, 1998). Nonetheless, variation in the rearing environment did affect some characters. A decrease in the number of nestlings in a brood resulted in increased body mass, total lipid mass, gizzard mass, body-size-adjusted resting $\dot{V}_{O_2}$ and the activity of HOAD in the heart.

Morphological response

Brood manipulation had no effect on the structural size of 16-day-old tree swallows (PC1 scores). This is consistent with previous studies on this species (Wiggins, 1990; Wheelwright et al., 1991). It could be argued that the addition or subtraction of a single nestling may have been insufficient to elicit a response. This is unlikely for two reasons. First, the addition of two nestlings (rather than one) exceeds the provisioning capacities of the parents in this population and frequently results in brood reduction (G. P. Burness, unpublished data). Second, at 16 days of age, individuals in enlarged broods had

smaller lipid stores and a lower body mass than those in reduced broods, suggesting that they were resource-limited.

Previous studies have shown that, under food restriction, other altricial species can maintain skeletal growth through catabolism of body tissues and lipid. Skeletal growth is significantly reduced, however, when energy reserves are depleted (Schew and Ricklefs, 1998). Although we detected few differences in organ and muscle mass as a consequence of treatment, individuals in the enlarged broods had significantly smaller lipid stores than those in the reduced broods. Rather than utilize stored energy to maintain growth, it is more likely, in the present study, that nestlings first met the energetic requirements for growth and maturation and then stored the remaining energy as lipid.

There were significant interannual differences in the composite index of size (PC1); at 16 days of age, nestlings were structurally larger in 1998 than in 1996. Systematic measurement error is unlikely because the same individuals (G. P. Burness and S. L. Wardrop) performed all measurements in both years. Interannual differences in morphology are more likely to be due to variation in weather conditions or food availability, as suggested previously for adults (Burness et al., 1998).

Phenotypic variation and developmental plasticity

During periods of reduced nutrition, nestlings of some species delay tissue maturation and feather growth or increase the duration of the nestling period (for a review, see Schew and Ricklefs, 1998). If this occurred in the present study, phenotypic values of characters at 16 days of age may have little similarity to the values of those same characters a few days later when individuals actually left the nest. We argue that this was unlikely.

There is a well-established negative relationship between the hydration state of a muscle and its mature function (mainly thermogenic capacities; e.g. Ricklefs and Webb, 1985). Water content, normalized to lean dry mass (a measure of protein content), typically decreases with a nestling's chronological age (Konarzewski, 1988). If nestlings in the enlarged broods were able to arrest their developmental program in response to unfavourable conditions, they would probably have had an increased muscle water content (less mature muscles) compared with nestlings of similar age from reduced broods. This was not observed (Table 1).

Although exact dates of nest departure are unknown, no difference in the duration of the nestling period has been reported previously in response to similar brood manipulations (Wheelwright et al., 1991). In addition, the duration of the nestling period is inversely related to the length of the ninth primary feather at 16 days of age (De Steven, 1980); the length of the primaries did not differ between treatments. Taken together, these data suggest that nestlings from each of the two treatments would have fledged at similar ages, with those from enlarged broods being in poorer condition for a given structural size.

Heat increment of feeding

Following ingestion of a meal, there is an unavoidable increase in metabolic rate, the heat increment of feeding (HIF). Conclusions drawn from our measurements of resting $\dot{V}_{O_2}$ rely on the assumption that, when nestlings in the two treatments were measured, they were of a similar absorptive state. Two lines of evidence indicate that this was the case. (i) Since the magnitude and duration of the HIF increase linearly with increasing meal mass (Chappell et al., 1997), we compared the mass of food in the small intestine of day 16 nestlings between treatments. Individuals in reduced broods did not have more food in their intestines (P>0.29): enlarged broods 0.15±0.05 g (N=7), reduced broods 0.12±0.06 g (N=7) (means ± S.D.). There was also no increase in resting $\dot{V}_{O_2}$ with increasing mass of intestine contents (P>0.69). Finally, we performed an ANCOVA with brood manipulation as a main effect and both intestine contents and PC1 as covariates. Nestlings in the reduced broods still had a significantly higher $\dot{V}_{O_2}$ than individuals in the enlarged broods ($F_{1,10}$=9.146, P=0.013); neither PC1 nor intestine contents was a significant covariate (P>0.10). (ii) In other passerines, there is a negative relationship between the duration of the HIF and nestling age (Chappell et al., 1997). If differences in $\dot{V}_{O_2}$ between treatments at 16 days of age were due primarily to an HIF, such differences should have been detectable on day 8. Contrary to the pattern seen on day 16, day 8 nestlings in the reduced treatment had on average a lower mass-specific $\dot{V}_{O_2}$ than individuals in the enlarged broods (although not significantly so, Fig. 1). Taken together, these points indicate that, if our measurements were affected by an HIF, both treatments were affected equally.

Phenotypic flexibility of organ size

The only organ that differed between brood sizes was the gizzard, being greater in individuals in the reduced broods. Since individuals in the reduced broods were presumably receiving more food, variation in the size of the muscular gizzard may be a result of an increased work load, analogous to a training effect (Piersma et al., 1993). For example, the gizzard of Japanese quail (*Coturnix japonica*) demonstrates rapid and repeated up- and down-regulation of size coincident with the fibre content of the diet (Stark, 1999). Captive red knots (*Calidris canutus*) also display phenotypic flexibility and decrease the size of their gizzard by approximately 75 % upon switching from small bivalves to soft food pellets (Dietz et al., 1999). Interestingly, following diet-switching experiments, Stark (1999) reported that the gizzard of quail never returned to the same level as that of unchallenged controls. Whether the differences we observed among nestling swallows are fixed is not known.

Implications for post-fledging survival

There is a well-established positive relationship between body mass at fledging and the probability of subsequent recruitment (e.g. Perrins, 1965; Tinbergen and Boerlijst, 1990; Both et al., 1999). Body mass may represent a general indicator of health (e.g. lipid stores) that allows for survival during periods of adverse weather (e.g. Perrins, 1965). However, although heavier individuals have larger fat stores, they are

often structurally larger and this may enhance survival by allowing them to dominate smaller individuals in competitive interactions (Garnett, 1981).

Apart from large lipid stores and structural size (although the latter was not observed in the present study), the role physiological factors play in determining the probability of survival and recruitment is unclear. An elevated size (or mass)-adjusted $\dot{V}_{O_2}$, although in itself presumably detrimental, has been linked to an elevated $\dot{V}_{O_2max}$ (aerobic capacity model; Bennett and Ruben, 1979). Recent avian studies have, however, failed to support such a correlation (Chappell et al., 1999; Hammond et al., 2000). An elevated resting $\dot{V}_{O_2}$ has also been linked with dominance status in some species (Røskaft et al.,1986; Bryant and Newton, 1994) but not others (Vézina and Thomas, 2000; Hammond et al., 2000). Even with an estimate of aerobic capacity or dominance, the consequence of an elevated resting $\dot{V}_{O_2}$ would be speculative without associated estimates of differential survivorship (e.g. Hayes and O'Connor, 1999).

The only enzyme showing a clear response to brood manipulation was HOAD. Individuals in the reduced broods had significantly higher cardiac HOAD activities, suggesting an increased capacity for oxidation of fatty acids. Mechanistically, this may be simply coupled to their elevated fat stores. For example, Marsh (1981) found a correlation between HOAD activity in the pectoral muscle and carcass fat levels in pre-migratory birds. During pre-migratory fattening, semipalmated sandpipers (*Calidris pusilla*) increase their capacity for fatty acid oxidation in skeletal muscle, but not the heart (Driedzic et al., 1993). Although the amount of fat in the diet can affect enzyme activities in both skeletal (Fisher et al., 1983) and cardiac (Power and Newsholme, 1997) muscle, nestlings presumably received diets of similar fat content. At present, the mechanisms underlying elevated heart HOAD activities are unclear.

In conclusion, manipulation of brood size early in ontogeny had minimal effects on the physiology and biochemistry of tree swallows shortly before fledging. A lack of response suggests that many characters may be relatively insensitive to environmental variation during development. Although some characters did respond, how they increase the probability of survival remains unknown.

This study would not have been possible without the field and laboratory assistance of J. Burns, A. Cosh, S. Dicken, D. Higgins, J. Lau, W. Park and C. Tucker. The staff of the Creston Valley Wildlife Management Area provided logistical support in the field. K. Campbell, M. Chappell, J. Christians, C. Guglielmo, S. Thornton, R. Ydenberg and an anonymous reviewer each read a previous version of the manuscript and provided suggestions for clarification. This research was funded by Natural Science and Engineering Research operating grants to P.W.H. and R. C. Ydenberg. G.P.B. and G.B.M. were supported by NSERC Post-Graduate Fellowships and University of British Columbia Graduate Fellowships.

References

Alisaukas, R. T. and Ankney, C. D. (1987). Age-related variation in the nutrient reserves of breeding American coots (*Fulica americana*). *Can. J. Zool.* **65**, 2417–2420.

Bennett, A. F. and Ruben, J. A. (1979). Endothermy and activity in vertebrates. *Science* **206**, 649–654.

Boag, P. T. (1987). Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). *Auk* **104**, 155–166.

Both, C., Visser, M. E. and Verboven, N. (1999). Density-dependent recruitment rates in great tits: the importance of being heavier. *Proc. R. Soc. Lond. B* **266**, 465–469.

Bryant, D. M. and Newton, A. V. (1994). Metabolic costs of dominance in dippers, *Cinclus cinclus*. *Anim. Behav.* **48**, 447–455.

Burness, G. P., Ydenberg, R. C. and Hochachka, P. W. (1998). Interindividual variability in body composition and resting rate of oxygen consumption in breeding tree swallows, *Tachycineta bicolor*. *Physiol. Zool.* **71**, 247–256.

Chappell, M. A., Bachman, G. C. and Hammond, K. A. (1997). The heat increment of feeding in house wren chicks: magnitude, duration and substitution for thermostatic costs. *J. Comp. Physiol.* B **167**, 313–318.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269–2279.

de Kogel, C. H. and Prijs, H. J. (1996). Effects of brood size manipulations on sexual attractiveness of offspring in the zebra finch. *Anim. Behav.* **51**, 699–708.

De Steven, D. (1980). Clutch size, breeding success and parental survival in the tree swallow (*Iridoprocne bicolor*). *Evolution* **34**, 278–291.

Dietz, M. W., Piersma, T. and Dekinga, A. (1999). Body-building without power training: endogenously regulated pectoral muscle hypertrophy in confined shorebirds. *J. Exp. Biol.* **202**, 2831–2837.

Dijkstra, C., Bult, A., Bijlsma, S., Daan, S., Meijer, T. and Zijlstra, M. (1990). Brood size manipulation in the kestrel (*Falco tinnunclus*): effects on offspring and parent survival. *J. Anim. Ecol.* **59**, 269–285.

Dobush, G. R., Ankney, C. D. and Krementz, D. G. (1985). The effect of apparatus, extraction time and solvent type on lipid extractions of snow geese. *Can. J. Zool.* **63**, 1917–1920.

Driedzic, W. R., Crowe, H. L., Hicklin, P. W. and Sephton, D. H. (1993). Adaptations in pectoralis muscle, heart mass and energy metabolism during premigratory fattening in semipalmated sandpipers (*Calidris pusilla*). *Can. J. Zool.* **71**, 1602–1608.

Fisher, E. C., Evans, W. J. and Phinney, S. D. (1983). Changes in skeletal muscle metabolism induced by a eucaloric diet. In *Biochemistry of Exercise* (ed. H. G. Knutgen and J. Poortmans), pp. 497–501. Champaign, IL: Human Kinetics.

Garland, T., Jr (1984). Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806–R815.

Garland, T. H., Jr, Bennett, A. F. and Daniels, C. B. (1990). Heritability of locomotor performance and its correlates in a natural population. *Experientia* **46**, 530–533.

Garnett, M. C. (1981). Body size, its heritability and influence on juvenile survival among great tits, *Parus major*. *Ibis* **123**, 31–41.

Gustafsson, L., Qvarnström, A. and Sheldon, B. C. (1995). Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature* **375**, 311–313.

Hammond, K. A., Chappell, M. A., Cardullo, R. A., Lin, R.-S. and

Johnsen, T. S. (2000). The mechanistic basis of aerobic performance variation in red junglefowl. *J. Exp. Biol.* **203**, 2053–2064.

Hayes, J. P. and O'Connor, C. S. (1999). Natural selection on thermogenic capacity of high altitude deer mice. *Evolution* **53**, 1280–1287.

Haywood, S. and Perrins, C. M. (1992). Is clutch size in birds affected by environmental conditions during growth? *Proc. R. Soc. Lond. B* **249**, 195–197.

Konarzewski, M. (1988). A model of growth in altricial birds based on changes in water content of the tissues. *Ornis Scand.* **19**, 290–296.

Koskela, E. (1998). Offspring growth, survival and reproductive success in the bank vole: a litter size manipulation experiment. *Oecologia* **115**, 379–384.

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343–348.

Marsh, R. L. (1981). Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the gray catbird (*Dumetella carolinensis*). *J. Comp. Physiol.* B **141**, 417–423.

McNamera, J. M. and Houston, A. I. (1992). State-dependent life history theory and its implications for optimal clutch size. *Evol. Ecol.* **4**, 143–147.

Mommsen, T. P. and Hochachka, P. W. (1994). Buffered salt solutions, culture media and tissue homogenization buffers. In *Biochemistry and Molecular Biology of Fishes*, vol. 6 (ed. P. W. Hochachka and T. P. Mommsen), pp. 649–657. Amsterdam: Elsevier.

Perrins, C. M. (1965). Population fluctuations and clutch-size in the great tit *Parus major*. *J. Anim. Ecol.* **34**, 601–647.

Piersma, T., Koolhaas, A. and Dekinga, A. (1993). Interactions between stomach structure and diet choice in shorebirds. *Auk* **110**, 552–564.

Power, G. W. and Newsholme, E. A. (1997). Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. *J. Nutrition* **127**, 2142–2150.

Richner, H. (1989). Habitat-specific growth and fitness in carrion crows (*Corvus corone corone*). *J. Anim. Ecol.* **58**, 427–440.

Ricklefs, R. E. and Webb, T. (1985). Water content, thermogenesis and growth rate of skeletal muscles in the European starling. *Auk* **102**, 369–376.

Robertson, R. J., Strutchbury, B. J. and Cohen, R. R. (1992). Tree swallow. In *The Birds of North America*, No. 11 (ed. A. Poole, P. Stettenheim and F. Gill). Philadelphia: The Academy of Natural Sciences.

Røskaft, E., Järvi, T., Bakken, M., Bech, C. and Reinersten, R. E. (1986). The relationship between social status and resting metabolic rate in great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*). *Anim. Behav.* **34**, 838–842.

Schew, W. A. and Ricklefs, R. E. (1998). Developmental plasticity. In *Avian Growth and Development: Evolution within the Altricial–Precocial Spectrum* (ed. J. M. Stark and R. E. Ricklefs), pp. 288–304. Oxford: Oxford University Press.

Schluter, D. and Gustafsson, L. (1993). Maternal inheritance of condition and clutch size in the collared flycatcher. *Evolution* **47**, 658–667.

Stark, J. M. (1999). Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes in organ size in response to changes in dietary fibre content. *J. Exp. Biol.* **202**, 3171–3179.

Tinbergen, J. M. and Boerlijst, M. C. (1990). Nestling weight and survival in individual great tits (*Parus major*). *J. Anim. Ecol.* **59**, 1113–1127.

Verhulst, S., Perrins, C. M. and Riddington, R. (1997). Natal dispersal of great tits in a patchy environment. *Ecology* **78**, 864–872.

Vézina, F. and Thomas, D. W. (2000). Social status does not affect resting metabolic rate in wintering dark-eyed junco (*Junco hymalis*). *Physiol. Biochem. Zool.* **73**, 231–236.

Wheelwright, N. T., Leary, J. and Fitzgerald, C. (1991). The costs of reproduction in tree swallows (*Tachycineta bicolor*). *Can. J. Zool.* **69**, 2540–2547.

Wiggins, D. A. (1990). Food availability, growth and heritability of body size in nestling tree swallows (*Tachycineta bicolor*). *Can. J. Zool.* **68**, 1292–1296.

Withers, P. C. (1977). Measurement of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and evaporative water loss with a flow through mask. *J. Appl. Physiol.* **42**, 120–123.