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PHYSIOLOGICAL AND BIOCHEMICAL CORRELATES OF BROOD SIZE AND ENERGY EXPENDITURE IN TREE SWALLOWS

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Summary

Intra-population variation in many fitness-related traits (e.g. clutch size) is often attributed to variation in individual parental quality. One possible component of quality is the level at which each individual can expend energy while provisioning dependent young. We used breeding tree swallows (*Tachycineta bicolor*) to test whether adults with large, natural-sized broods and/or nestlings in good nutritional condition had relatively high daily energy expenditures (*DEE*s). Adults with high *DEE*s were predicted to have large internal organs and high metabolic capacities. We first measured the growth rate of nestlings in natural broods of five, six and seven over a 4-day period and then measured parental *DEE* using doubly labelled water. Adults were then dissected for analyses of body composition and to determine maximum enzyme activities in the pectoral muscle. Although the total mass gain of large broods was greater than that of small broods, parental *DEE* was independent of brood size. We hypothesize that adults matched their clutch size (and consequently, brood size) to their individual foraging efficiencies. When statistically controlling for the effects of brood size, in one of two years there was a positive correlation between *DEE* and brood mass. This suggests that among individuals rearing the same-sized broods there were reproductive benefits of a relatively high *DEE*. There was no correlation between either brood size or *DEE* and the mass of any internal organ or the metabolic capacity of the pectoral muscle.

Key words: energetics, doubly labelled water, daily energy expenditure, parental care, clutch size, fitness, performance, provisioning, enzyme activity, tree swallow, *Tachycineta bicolor*.

Introduction

Within many populations of passerine birds, there is considerable variation in clutch size, despite the fact that individuals laying the largest number of eggs often raise the most recruits (e.g. Boyce and Perrins, 1987). One hypothesis to explain the existence of this variation proposes that females adjust the size of their clutch to their own individual circumstances (individual optimization hypothesis; Perrins and Moss 1975), for example, to the quality of their territory (Högstedt, 1980) or to their own individual abilities. Although territory quality can be defined in terms of ecological variables such as predation risk or food resources, variables defining individual quality are much less clear.

The provisioning of dependent nestlings requires an elevation of parental activity over that of non-breeding adults, and consequently, an elevation of an individual's daily energy expenditure (*DEE*). If parental effort is energetically costly, individuals capable of sustaining higher energy expenditures may lay larger clutches and/or produce offspring in better nutritional condition (Moreno et al., 1997). There is a positive correlation between offspring condition and subsequent recruitment (e.g. Tinbergen and Boerlijst, 1990), so this provides a plausible link between parental energy expenditure and fitness.

In the same way that inter-individual variation exists in the capacity for short-term energy expenditure (aerobic capacity, $\dot{V}_{O_2max}$; Chappell et al., 1999; Hammond et al., 2000), individual variation in *DEE* has also been reported (e.g. Bryant, 1991; Konarzewski and Diamond, 1994; Moreno et al., 1997). To attain a high *DEE*, however, individuals probably require various physiological adaptations (e.g. Daan et al., 1990). Studies of *DEE* in laboratory mice have shown, for example, that individuals with relatively high rates of energy intake (a surrogate of *DEE*) had relatively large kidneys and intestines (Konarzewski and Diamond, 1994). The organs of the abdominal cavity account for a large fraction of the mass-specific resting metabolic rate, so individuals with relatively large kidneys had increased energy expenditure while at rest. This results in a hypothesized trade-off between the potential benefits of attaining a high *DEE* and the costs of maintaining the organs necessary to do it (Kersten and Piersma, 1987; Daan

et al., 1990; Hammond and Diamond, 1997). Despite the appeal of such a hypothesis, empirical support is weak (Ricklefs et al., 1996, Meerlo et al., 1997, Speakman, 2000).

Two recent avian studies have shown that measurements of both resting metabolic rate and *DEE* are repeatable between breeding seasons (Bech et al., 1999; Potti et al., 1999). The stability of energy expenditure over relatively long periods suggests that it may be possible to define individual quality within a physiological or metabolic context. Even though many physiological traits display considerable phenotypic flexibility (Piersma and Lindström, 1997), they probably still retain some genetic variance (e.g. Garland et al., 1990). Identification of the physiological or biochemical correlates of *DEE* may give insight into traits that would be subject to potential evolutionary change under selection for whole-animal performance.

In an attempt to understand better the physiological causes and ecological consequences of variation in individual quality, we studied breeding tree swallows, *Tachycineta bicolor*. Because tree swallows are aerial insectivores and do not hold feeding territories, we assumed that all individuals experienced similar food availability (Hussell and Quinney, 1987). Tree swallows are single-brooded, so potential intra-seasonal trade-offs between the size of the first and second broods are avoided (e.g. Verhulst, 1995).

We asked three primary questions. (i) Do parents rearing large natural-sized broods trade off nestling quality for quantity? (ii) Does parental *DEE* correlate with indices of fitness (natural clutch size and nestling mass)? (iii) What are the physiological and biochemical correlates of parental *DEE*, and do these differ among adults rearing different-sized broods?

Materials and methods

Study area and selection of study nests

The field component of this study was performed in May and June 1996 and 1997 at the Creston Valley Wildlife Management Area, near Creston, British Columbia, Canada. Approximately 180 nest boxes were erected, 15–20 m apart, along man-made dikes within the Management Area.

Beginning in the first week of May, boxes were checked daily for signs of breeding by tree swallows and the presence of eggs. Females lay a single egg per day, typically on consecutive days, until clutch completion. In this population, the maximum clutch size is eight, with a modal clutch of six eggs. Clutch completion is followed by 14–15 days of incubation (Robertson et al., 1992). To minimize disturbance, no nest checks were conducted during incubation. Within 1–2 days of predicted hatch dates, nest checks were resumed (hatch = day 1). Within a clutch, hatching was relatively synchronous and was typically complete within 1–2 days. First-time breeding females were identifiable on the basis of plumage (1 year old; Hussell, 1983) and were excluded from the present study. In females older than 2 years, the confounding effects of age and breeding experience on clutch size are minimal (Robertson et al., 1992).

In both 1996 and 1997, egg-laying began during the first week of May and continued into early June. To minimize the possibility of including females laying replacement clutches, we only considered nests with clutches initiated in May. Study nests were chosen on the basis of their original clutch size (five, six or seven eggs). To minimize date as a correlate of clutch size, we randomized the choice of study nests across each breeding season (i.e. not all seven-egg nests were selected early in the season).

Nestling mass and growth rate

On day 4, nestlings from each study nest were weighed using a spring-loaded balance (±0.5 g) and banded loosely. If a nestling was too small to be banded, it was marked with indelible marker and banded within a few days. On day 8, nestlings were reweighed and the bands tightened. Eggs hatch relatively synchronously. Consequently, if an egg had failed to hatch by day 4 it was replaced by a 4-day-old nestling (±1 day) from another nest. In this way, brood size remained equal to the original clutch size. If a nestling died between days 4 and 8, it was replaced by a nestling of similar age to maintain the original brood size. However, no measure of growth for that brood was recorded.

Nestlings whose parents were involved in a study of energetics (below) were weighed a third time on day 9. This third weighing was used as an indirect measure of whether parents were behaving normally following injection and release on day 8 (i.e. did nestlings lose weight over the duration of the energetic study).

Doubly labelled water

We measured the rates of CO_2 production ($\dot{V}_{CO_2}$) of tree swallows rearing natural broods of five, six or seven nestlings using the doubly labelled water technique (*DLW*; Lifson and McClintock, 1966). To standardize for brood age, all adults were captured at the nest box on day 8 of chick rearing. In broods of five and seven, we attempted to capture both members of the pair; in broods of six, a single parent was captured.

The *DLW* injection solution was prepared by mixing 120 µl of ^3H_2O (110.6 MBq) with 8.97 ml of $H_2{}^{18}O$ (97 atom %). Using a calibrated glass syringe, 100 µl of solution (approximately 1.22 MBq of tritium per individual) was injected into the pectoral muscle of each adult. Each adult was then weighed using a spring-loaded balance (±0.5 g), banded, and held for 1 h in an individual brown paper bag to allow for equilibration of the isotopes with the body water (e.g. Williams and Nagy, 1984). Following equilibration, we collected approximately 150 µl of blood from the brachial vein into heparinized microcapillary tubes and then released the bird near the site of capture. After approximately 24 h, the bird was recaptured, and a second set of blood samples was taken from the other wing. In each year, two non-experimental females were captured at the study site, and a blood sample was taken to determine background levels of ^{18}O and ^3H.

In 1996, microcapillary tubes containing blood samples

were immediately flame-sealed in the field using a butane torch. In 1997, tubes were first sealed with Critocaps, and then flame-sealed upon return to the laboroatory at the end of the day. All blood samples were stored at 4 °C until distillation and analysis by Dr K. A. Nagy's Laboratory of Biomedical and Environmental Sciences, UCLA. ^{18}O concentration was measured in triplicate using cyclotron-generated proton activation analysis. ^{3}H activity was measured in duplicate using a liquid scintilation counter.

Adults rearing six nestlings were released following the second blood sample. To investigate the physiological and biochemical correlates of clutch size and energy expenditure, adults rearing either five or seven nestlings were killed (see below). Their nestlings were distributed among non-study nests in the population.

Environmental temperature

The daily maximum and minimum air temperatures during the study period were obtained from an Atmospheric Environment Service weather station, approximately 5 km from the study site. As most adults were captured and injected between 10:00 and 13:00 h, the maximum temperature experienced during the *DLW* trial was assumed to occur on the day of capture (nestling day 8). The minimum temperature probably occurred between approximately 00:00 and 05:00 h and was considered to be the lowest temperature recorded for the day of recapture (nestling day 9).

Haematocrit and haemoglobin

We collected an additional 100–200 µl blood sample from adults rearing either five or seven nestlings. To determine haematocrit (Hct, %), microcapillary tubes containing the samples were centrifuged at maximum speed for 10 min using an Adams micro-haematocrit bench-top centrifuge, and the percentage of the tube occupied by packed cells was then measured. The concentration of haemoglobin ([Hb], $g\,dl^{-1}$) was determined using a portable HemoCue B-Hemoglobin photometer (Ängelholm, Sweden). The number of replicates for each blood variable was determined by the size of the blood sample and ranged from one to three (which were averaged).

For five individuals in which [Hb] but not Hct was measured, we estimated Hct from regressions of Hct on [Hb] for the 29 individuals in which both variables had been measured. Separate predictive equations were necessary for each year because the slopes of the regression lines differed (ANCOVA, $P<0.15$). As Hct and [Hb] are strongly correlated ($r=0.83$, $P<0.001$, $N=29$), we present only the results for Hct.

Body composition

During two breeding seasons, immediately following blood sampling we killed 49 adults (29 females and 20 males) rearing either five or seven nestlings (following the guidelines of the Canadian Committee on Animal Care). Within 1–2 min of death, a sub-sample (approximately 300 mg) of the right pectoralis major was removed from each individual and immediately frozen in a liquid-N_2-charged dry shipper for enzyme assays. The remainder of the pectoralis and supracoracoideous (hereafter, 'pectoralis') was then removed, followed by the heart, liver, small intestine, gizzard and kidney. All tissues except the gizzard were stored in air-tight cryovials and frozen in the dry shipper. Each carcass (including the gizzard) was double-bagged and stored at −20 °C. Upon return from the field site, we transferred the sub-samples of pectoralis to liquid N_2 and stored the remainder of the tissues and carcass at either −20 °C or −80 °C.

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

Determination of lipid mass was restricted to the carcass and pectoralis. In preparation for fat extraction, carcasses were partially thawed, plucked of all feathers and weighed (to ±0.0001 g). The carcass and pectoralis were dried to constant mass in an oven at 70 °C (carcass) and freeze dryer (pectoralis). These dried samples were then fat-extracted for 7 h in a Soxhlet apparatus containing petroleum ether as the solvent (Dobush et al., 1985). Following extraction, the carcass and pectoralis 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 represented the mass of lipid.

Muscle biochemistry

The sub-samples (approximately 300 mg) of the pectoralis major were removed from liquid N_2, weighed frozen (to ±0.0001 g) and homogenized in a glycerol buffer, as described previously (Burness et al., 2000). Homogenates were stored at −80 °C until they were assayed (maximum 4 months). This homogenization buffer allows samples to be frozen for extended periods with no loss of enzyme activity (Mommsen and Hochachka, 1994).

As an index of the maximum capacity for flux through specific steps in various metabolic pathways, we measured the maximum catalytic activity (V_{max}) under optimal conditions of the following enzymes: lactate dehydrogenase (index of anaerobic glycolysis), 3-hydroxyacyl-CoA dehydrogenase (index of fatty acid catabolism), citrate synthase (index of aerobic capacity) and pyruvate kinase (index of glycolytic capacity).

All assays were performed on a temperature-controlled six-cuvette spectrophotometer (Perkin Elmer Lamda 2). Assay temperature was maintained at 42 °C using a Lauda RM6 circulating water bath and water-jacketed cuvette holders. In all assays, uncentrifuged homogenates were used to avoid potential loss of activity in the pellet. Each reaction was replicated in three cuvettes. Values for the two cuvettes with the most similar activity were averaged; in cases where values from the three cuvettes were equidistant, all three were averaged. Preliminary experiments confirmed that levels of all

substrates and cofactors were saturating but not inhibitory. With the exception of citrate synthase, all assays were at pH 7.0 and 340 nm. Citrate synthase was assayed at pH 8.0, 412 nm. Enzyme 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. Lactate dehydrogenase (EC 1.1.1.27; LDH): 50 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN, 1.0 mmol l^{-1} pyruvate. 3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35; HOAD): 50 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN, 0.05 mmol l^{-1} acetoacetyl CoA. Citrate synthase (EC 4.1.3.7; CS): 50 mmol l^{-1} Tris buffer, 0.05 % Triton X-100, 0.2 mmol l^{-1} 5,5′dithiobis(2-nitrobenzoic acid) (DTNB), 0.2 mmol l^{-1} acetyl CoA, 0.5 mmol l^{-1} oxaloacetate (omitted from the control cuvette). Pyruvate kinase (EC 2.7.1.40; PK): 50 mmol l^{-1} imidazole, 0.15 mmol l^{-1} NADH, 10 mmol l^{-1} β-mercaptoethanol, 1.0 mmol l^{-1} NaCN, 100 mmol l^{-1} KCl, 10 mmol l^{-1} $MgCl_2$, 10 μmol l^{-1} fructose 1,6-bisphosphate, 5.0 mmol l^{-1} ADP, 5 mmol l^{-1} phosphoenolpyruvate, excess LDH (approximately 5 i.u. ml^{-1}). To account for PK contamination in the coupling enzyme (LDH), we performed six additional control reactions containing no homogenate. The mean control rate was subtracted from all PK reaction rates before calculating enzyme activity. Additional control reactions (containing no substrate) were initially run for each enzyme. The rates of control reactions were low for all enzymes except CS and were subsequently omitted.

Calculations of daily energy expenditure

An individual's initial total body water pool was estimated using the ^{18}O dilution space, calculated according to Appendix I of Nagy (Nagy, 1983). On occasion, a small amount of water remained on the skin following an injection. Because of the occasional uncertainty concerning the exact amount of water that was injected, instead of individual values, a mean percentage body water of 66.2 % was assumed in all cases. This percentage estimate was based on the mean dilution space calculated for 43 individuals with 'clean' injections. Rates of CO_2 production ($\dot{V}_{CO_2}$) were calculated using equation 1 of Nagy (Nagy, 1983).

We converted $\dot{V}_{CO_2}$ to *DEE* assuming 26.2 J ml^{-1} CO_2 for insectivorous food (Weathers and Sullivan, 1989). The rate of CO_2 production of individuals that lost more than 4 % of their mass during the experiment (N=6) was corrected (following Weathers and Sullivan, 1989). This resulted in a marginal increase in estimates of their *DEE* (by 0.84±0.16 %, N=6). One swallow increased in mass by 0.78 g, representing a gain of 4.2 %. Changes in body composition between release and recapture were unknown, so we made no attempt to correct this individual's *DEE* for mass gain.

Sixty-one adult tree swallows were successfully recaptured, and of these, 46 yielded reliable estimates of *DEE* during provisioning. Estimates of *DEE* were considered to be unreliable and were excluded from analyses either if the final ^{3}H or ^{18}O values had decayed to background (N=4) or if the estimated *DEE* was less than the allometrically predicted basal metabolic rate, *BMR* (N=7, from Burness et al., 1998). Values for four additional adults were omitted because their *DEE* was less than 1.5 times *BMR* (unlikely in an aerial insectivore provisioning young; Williams, 1988) and/or their nestlings displayed a large mass loss during the trial, suggesting potential negative effects of the injection on the parents. Although we recognize that adults with *DEE*<1.5*BMR* may simply have low energy expenditures, we considered it more likely that these individuals ceased foraging following injection.

Statistical analyses

Data were transformed as necessary to meet the assumptions of multivariate statistical tests. Data that did not meet these assumptions and could not be transformed were analyzed using non-parametric tests. The influence of potential covariates (e.g. time, date, body mass), main effects (e.g. year, sex or brood size) and interaction terms on dependent variables were first explored using either a forward or backward stepwise regression. Probabilities for inclusion and exclusion were set at 0.05 and 0.10 respectively. Terms significant in the stepwise regression were then included in one- or two-way analysis of variance (ANOVA), analysis of covariance (ANCOVA) or multiple regressions. Interaction terms were excluded from models when P>0.15. For results to be viewed graphically, we analyzed residuals on occasion. In these cases, P-values were corrected to account for the degrees of freedom lost in the generation of the residuals (Hayes and Shonkwiler, 1996).

Whenever possible, the year and sex of the parents were pooled (with either year or sex included as a main effect). However, in 10 cases, the male and female were captured from the same nest; consequently, in all analyses involving brood size or nestling mass and growth rate, sexes were considered separately.

To avoid the possibility of spurious autocorrelation in analyses of body composition, the mass of each organ was subtracted from total body mass before each computation (Christians, 1999). Unless noted otherwise, data are reported as least-squares means ±1 standard error of the mean (S.E.M.) and probabilities are two-tailed. Statistical significance was claimed at P<0.05. Most analyses used JMP statistical software; power analyses were performed using PASS 2000.

Results

Brood size and nestling growth rate

Growth was followed in 52 unmanipulated nests between days 4 and 8 (22 in 1996; 30 in 1997). The total mass gain of broods per day (brood growth rate) increased with increasing natural brood size ($F_{2,49}$=41.48, P<0.001; all brood sizes differed significantly, Tukey HSD P<0.05; Fig. 1). There was a marginally significant difference in the growth rate of

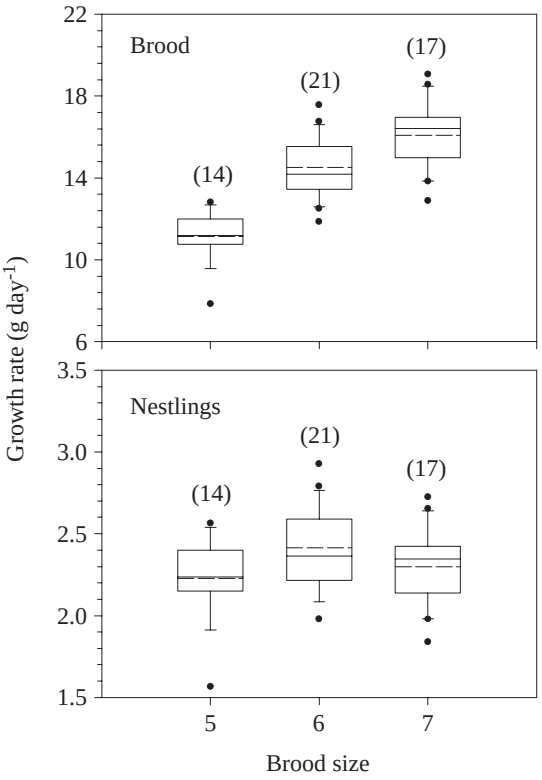

Fig. 1. Box plots of growth rates between days 4 and 8 of entire tree swallow broods and of individual nestlings. The solid horizontal line in the middle of each box is the median, the dashed line is the mean. The bottom and top of the boxes are the 25th and 75th percentiles. The vertical lines above and below the boxes are the 10th and 90th percentiles; filled circles indicate data points falling outside of this range. Sample sizes are given in parentheses.

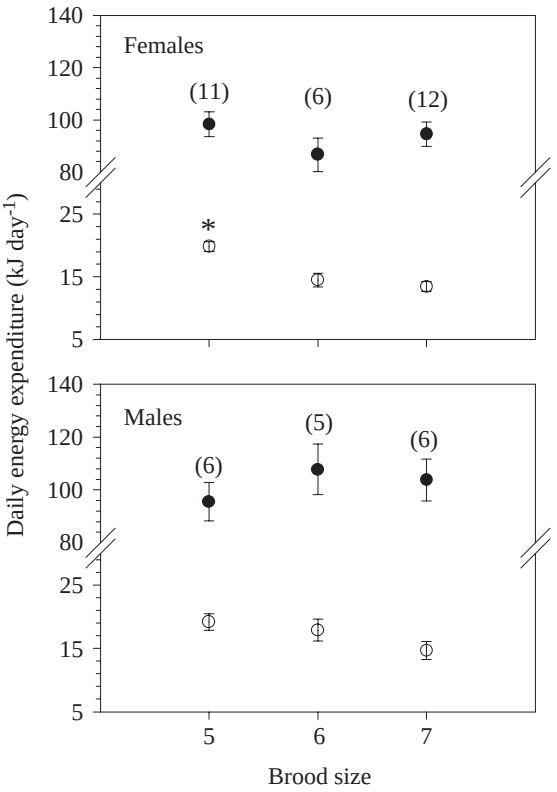

Fig. 2. Daily energy expenditure of adult tree swallows rearing natural-sized broods. Filled circles, total parental energy expenditure; open circles, energy expenditure expressed as investment per nestling. Values are least-squares means ±1 S.E.M., sample sizes are given in parentheses. An asterisk denotes a significant difference (P<0.05) between females raising broods of five and females raising broods of six or seven.

individual nestlings across brood size (P=0.054, Fig. 1). Contrary to expectations, the lowest mean growth rates were found in nestlings from broods of five. Growth rates did not differ between years (P>0.10).

Potential correlates of DEE

Various factors influence estimates of *DEE* (e.g. body mass). These need to be identified (and controlled for) before relationships among *DEE*, brood size and nestling growth can be determined (Speakman, 1997).

The *DEE* of adult tree swallows ranged from 56.1 to 136.3 kJ day^{-1}, with an average value of 101.0±18.8 kJ day^{-1} (mean ± S.D., N=46) (Table 1). The *DEE* of males and females did not differ (P>0.10). In nests in which both individuals were captured, there was no correlation between male and female *DEE* (P>0.50, N=10).

The mean *DEE* of individuals in 1996 was less than that in 1997 (86.7±24.3 kJ day^{-1}, N=13, *versus* 106.6±12.6 kJ day^{-1}, N=33; Z=–2.68, P<0.01). There was a weak but significant increase in *DEE* with increasing body mass (controlling for year: $F_{1,43}$=4.55, P<0.05, partial r^2=0.10). This correlation did not exist, however, when years were analyzed separately (P>0.05). Adults lost on average 0.16±0.53 g day^{-1} (mean ± S.D., N=42). An individual's change in body mass was not a significant predictor of its *DEE* (controlling for body mass and year: P>0.50).

The mean elapsed time between release and recapture was 1482±131 min (mean ± S.D., N=46; range 1124–1729 min). There was no correlation between *DEE* and the deviation of the recapture interval from 24 h (recapture interval minus 24 h; controlling for body mass and year: $F_{1,42}$=0.42, P>0.50).

Daily minimum temperature was not correlated with *DEE* in either 1996 or 1997 (P>0.15). In 1997 only, there was a marginally significant decrease in *DEE* with increasing daily maximum temperature (r=–0.31, N=33, P=0.079).

Parental DEE, brood size and nestling growth rates

Brood size

Parental *DEE* was independent of brood size (P>0.35, Fig. 2). The lack of significance was probably not due to insufficient statistical power. From Fig. 1, we estimated that broods of seven nestlings had an approximately 30 % greater mass gain per day than broods of five nestlings. Consequently, we predicted *a priori* that the *DEE* of adults rearing seven nestlings would be approximately 30 % higher

Table 1. *The rate of CO_2 production and the estimated daily energy expenditure of male and female tree swallows rearing natural-sized broods*

Nest identity	Year	Sex	Brood size	Mass[a] (g)	Mass change (g day^{-1})	$\dot{V}_{CO_2}$[b] (ml day^{-1})	*DEE*[c] (kJ day^{-1})
2A-41	1996	M	5	19.38	–0.23	2179.7	57.1
D2-31	1996	M	5	18.63	–0.27	4614.6	120.9
D2-01	1997	M	5	20.63	+0.22	3907.7	102.4
2A-16	1997	M	5	18.63	–0.26	3830.5	100.4
2A-57	1997	M	5	19.50	0	4185.9	109.7
2A-63	1997	M	5	18.25	–0.48	3696.4	96.9
D2-20	1997	M	6	21.13	–0.24	4201.2	110.1
D2-32	1997	M	6	19.50	...[d]	4676.6	122.5
2A-14	1997	M	6	18.13	–0.25	3931.1	103.0
2A-56	1997	M	6	19.88	+0.25	4567.9	119.7
2A-76	1997	M	6	17.38	+0.25	4556.4	119.4
2A-77	1996	M	7	21.64	–0.62	3635.9	95.3
D2-12	1997	M	7	20.00	–0.61	3883.5	101.8
D2-44	1997	M	7	18.88	+0.74	3306.2	86.6
2A-17	1997	M	7	18.75	+0.46	4260.2	111.6
2A-21	1997	M	7	19.75	+0.46	4579.7	120.0
2A-78	1997	M	7	18.88	+0.25	5203.0	136.3
2A-59	1996	F	5	19.88	–1.24	4487.9	118.5
2A-41	1996	F	5	21.50	0	3804.0	99.7
D2-25	1996	F	5	18.75	...	2515.3	65.9
D3-15	1996	F	5	18.00	0	3622.3	94.9
2A-16	1997	F	5	17.50	0	4209.0	110.3
2A-57	1997	F	5	18.25	–0.51	3908.9	102.4
2A-63	1997	F	5	16.50	–0.49	4177.1	109.4
D2-01	1997	F	5	19.00	+0.47	4578.8	120.0
D2-11	1997	F	5	16.75	–0.48	3691.3	96.7
D2-21	1997	F	5	19.00	–0.46	3716.8	97.4
D2-29	1997	F	5	17.25	–0.54	4147.9	108.7
2A-37	1996	F	6	17.25	+0.42	3406.7	89.3
D3-09	1996	F	6	19.25	...	2141.5	56.1
2A-07	1997	F	6	18.00	0	4310.9	113.0
2A-25	1997	F	6	19.63	–0.23	4092.4	107.2
2A-33	1997	F	6	18.00	–1.06	3218.6	85.1
2A-73	1997	F	6	18.63	+0.78	3754.3	98.4
2A-40	1996	F	7	17.38	–0.24	3352.5	87.8
2A-77	1996	F	7	19.10	...	4551.0	119.2
D2-24	1996	F	7	16.63	–0.70	2205.6	58.3
D2-16	1996	F	7	17.50	0	2449.3	64.2
2A-17	1997	F	7	17.13	–1.25	3583.7	94.8
2A-21	1997	F	7	17.13	+0.23	3713.2	97.3
2A-78	1997	F	7	18.25	–1.00	4683.6	123.4
2A-80	1997	F	7	20.13	+0.25	4616.1	120.9
D3-01	1997	F	7	17.75	+0.46	3397.7	89.0
D3-16	1997	F	7	19.25	0	4641.4	121.6
D2-06	1997	F	7	17.25	+0.42	3759.4	98.5
D2-12	1997	F	7	18.50	–1.21	3169.5	83.9

[a]Average of initial and final mass.
[b]Rate of carbon dioxide production.
[c]Daily energy expenditure.
[d]Either the initial or final mass was missed.

than that of those rearing five nestlings. We had a power of 0.80 to detect a 25 % difference in energy expenditure among females rearing each of the three brood sizes and the ability to detect a 36 % difference among males. Finally, if the sexes were pooled, parental *DEE* remained independent of brood size (controlling for year, mass and sex of parent: $P>0.90$)

despite the ability to detect a 22 % difference among means (at a power of 0.80).

When parental DEE was expressed per nestling rather than per brood, females rearing broods of five spent significantly more energy per nestling than females rearing broods of six or seven ($F_{2,24}$=18.64, P<0.001; Tukey HSD P<0.05; Fig. 2). In males, parental energy expenditure per nestling was independent of brood size (P=0.066, Fig. 2). If years were considered separately, males in 1997 rearing broods of five or six nestlings spent more energy per nestling than males rearing broods of seven ($F_{2,11}$=7.13, P<0.05; Tukey HSD P<0.05). *DEE* in 1996 was measured in only three males (Table 1), precluding a separate analysis.

Nestling mass and growth rate

Parental *DEE* was analyzed with respect to the mass gain of an individual's brood between days 4 and 8 and with respect to the total mass of the brood on day 8. Years and sexes were analyzed separately because of significant interaction terms. As we predicted positive relationships among variables, *a priori*, *P*-values are one-tailed.

In 1997, females with a high *DEE* between days 8 and 9 reared broods that had previously displayed a high growth rate between days 4 and 8 (controlling for brood size: $F_{1,13}$=10.83, P<0.01; Fig. 3A). A single nest with a high Studentized residual (–2.89) was omitted from the analysis; the nestlings from this nest had the highest body mass on day 4 (>2.5 S.D. from the mean) and showed little mass gain between days 4 and 8. Male *DEE* was not correlated with the previous mass gain of his brood (controlling for brood size: P=0.122, Fig. 3A). When controlling for brood size, the *DEE* of both males and females increased with increasing brood mass on day 8 (females: $F_{1,14}$=21.40, P<0.001; males: $F_{1,9}$=4.57, P<0.05; Fig. 3B).

In 1996, there was no correlation between female *DEE* and the mass gain of her brood between days 4 and 8 (controlling for brood size: P>0.80). The correlation between female *DEE* and brood mass on day 8 was more complex than in 1997 and varied with brood size (interaction between *DEE* and brood size, P<0.01). In females rearing broods of five or six nestlings, there was a marginally significant increase in *DEE* with increasing brood mass on day 8 (P=0.089, N=5 nests). In contrast, females rearing seven nestlings showed a decline in *DEE* with increasing brood mass (r=–0.98, P<0.05, two-tailed test, N=4 nests). Only three males were labelled in 1996, so analysis was not possible.

Relationships among growth, brood mass and parental DEE were not driven by covariation with ambient temperature (*sensu* Dykstra and Karasov, 1993). There was no correlation between temperature on day 8 (maximum or minimum) and either mass gain of the brood between days 4 and 8 or total brood mass on day 8 in either year (controlling for brood size: P>0.05).

Physiological correlates of brood size and DEE

Adult organ and tissue masses were unrelated to brood size (P>0.10), but differed between years (Table 2). In females, the mean mass of the pectoralis and kidney were, respectively, 8 % and 15 % greater in 1997 than in 1996 (controlling for body mass: pectoralis, $F_{1,24}$=7.30, P<0.05; kidney, $F_{1,23}$=8.78). In males, mean liver mass was 35 % greater in 1997 than 1996 (controlling for body mass: $F_{1,14}$=5.86, P<0.05). No other organs showed inter-annual differences. The mass of stored lipid was independent of brood size and year of study in both sexes (controlling for lean body mass, date and time of capture: P>0.20, Table 2).

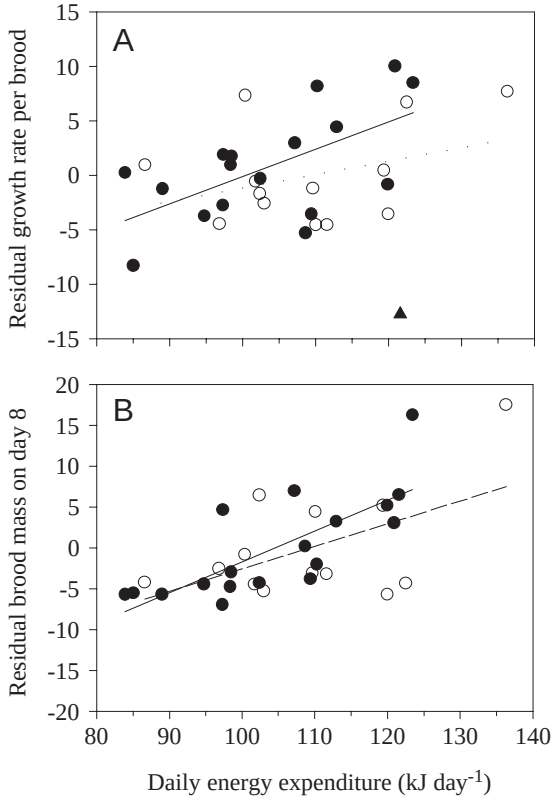

Fig. 3. Daily energy expenditure of adult tree swallows in 1997 and (A) the residual growth rate of their broods between days 4 and 8 and (B) the residual mass of their broods on day 8. Residuals controlled for the effect of brood size on the dependent variable. Females, filled circles, solid line; males, open circles, dashed line. The filled triangle was an outlier (see text) and was omitted from analyses. All correlations are significant (P<0.05), except for that of males in A (P=0.12).

The mean Hct of adult tree swallows was 43.4±5.1 % (mean ± S.D., N=46) and ranged from 33.2 to 54.3 %. Hct was independent of brood size, year of study and sex (P>0.20). Females captured later in the season had a significantly lower Hct than those captured early ($F_{1,23}$=6.07, P<0.05); this relationship was not seen for males (P>0.40).

To determine whether variation in parental *DEE* was related to variation in the mass any organ or tissue, we performed multiple regressions and generated residuals. Residuals of *DEE* did not correlate with the residuals of any organ or tissue (P>0.30). In an attempt to generate a predictive equation for

Table 2. *Body composition of adult tree swallows*

Organ/tissue	*N*	Brood size		*P*-value	
		Five	Seven	Brood size	Year[a]
Females					
Pectoralis	14, 14	2.62±0.05	2.62±0.05	0.979	0.012
Heart	15, 14	0.23±0.01	0.23±0.01	0.643	0.562
Kidney	14, 14	0.25±0.01	0.25±0.01	0.811	0.007
Liver	15, 13	0.60±0.02	0.64±0.02	0.153	0.057[b]
Intestine	15, 13	0.68±0.02	0.69±0.03	0.865	0.162
Gizzard	14, 14	0.46±0.02	0.43±0.02	0.302	0.224
Lipid	14, 14	0.51±0.06	0.47±0.04	0.491	0.229
Males					
Pectoralis	11, 9	2.71±0.12	2.77±0.12	0.722	0.084
Heart	11, 9	0.25±0.01	0.24±0.01	0.527	0.850
Kidney	11, 9	0.25±0.01	0.26±0.01	0.671	0.203
Liver	10, 9	0.65±0.04	0.58±0.04	0.249	0.030
Intestine	11, 9	0.75±0.03	0.71±0.03	0.350	0.118
Gizzard	10, 8	0.45±0.03	0.42±0.03	0.605	0.090
Lipid	10, 9	0.55±0.04	0.51±0.08	0.504	0.977

Values are least-squares means ± S.E.M. (standard error of mean) from ANCOVA; lipid masses are means ± 1 S.E.M.

Masses are in grams.

Sample sizes (*N* values) varied across organs and tissues because of missing data.

In analyses of females, the pectoralis, kidney and gizzard each had a single outlier with a large Studentized residual (>3.0), which has been omitted.

[a]When a significant difference occurred between years, 1997>1996.

[b]Significant year × time of capture interaction.

DEE, we performed a backward stepping multiple regression. Residuals of *DEE* could not be predicted by the residuals of any organ or tissue.

Biochemical correlates of brood size and DEE

The only enzyme that demonstrated a significant allometric scaling with body mass was pyruvate kinase: $\log_{10}(\text{PK activity}) = -0.54\log_{10}(\text{mass}) + 3.54$ (r^2=0.15, N=33, P<0.05). In contrast to previous studies of other taxa (e.g. fish, Burness et al., 1999, and references within), the slope of the allometric relationship was negative rather than positive. Despite considerable variance in pectoral muscle enzyme activity, activity was unrelated to brood size (P>0.20; Table 3) and parental *DEE* (P>0.30).

Discussion

This study of natural brood size variation in tree swallows demonstrated (i) that adults did not trade off nestling quality for quantity, (ii) that parental *DEE* and brood size were unrelated, (iii) that parental *DEE* and brood mass were positively related (in one year) and (iv) that adult body composition and muscle biochemistry were unrelated to brood size or parental *DEE*.

Parental effort: brood size and DEE

The growth rate of individual nestlings in large natural broods was the same as in small natural broods, indicating that parents did not trade off nestling quality for their quantity (Fig. 1). Although energetic savings resulting from decreased heat loss per nestling in large broods may have played a role in the observed patterns (Royama, 1966), experimental brood enlargements in this same population suggest that this was not the case. Individual nestlings in artificially enlarged broods

Table 3. *Enzyme activity in the pectoralis muscle of adult tree swallows*

Enzyme	Brood size		*t*	*P*-value
	Five	Seven		
Females				
PK	761.7±69.84	736.4±77.00	0.71	0.491
CS	277.6±31.62	284.5±39.59	0.39	0.701
HOAD	115.1±18.80	117.8±23.76	0.26	0.802
LDH	349.4±41.44	326.2±31.90	1.30	0.213
Males				
PK	696.7±80.82	669.5±23.59	0.86	0.405
CS	288.6±39.84	276.1±22.38	0.74	0.473
HOAD	117.2±18.7	131.3±27.39	1.22	0.243
LDH	350.3±77.7	364.0±49.79	0.41	0.690

Enzyme activities are in i.u. g^{-1} tissue, expressed as mean ± 1 S.D.

Sample sizes: females, five chicks (N=9), seven chicks (N=8); males, five chicks (N=9), seven chicks (N=7).

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

have lower growth rates than either controls (G. P. Burness, unpublished data) or broods with a reduced number of nestlings (Burness et al., 2000). This is inconsistent with an energetic savings in larger broods.

Despite differences among broods of different size in total mass gain, parental energy expenditure was independent of brood size (Fig. 2). In fact, when energy expenditure was expressed as investment per nestling, individuals rearing broods of seven spent less energy than those rearing broods of five. One explanation is that individual adults differed in their foraging efficiency, with the most efficient adults rearing the largest broods. Variation in efficiency has been demonstrated previously in European kestrels (*Falco tinnunculus*): males with large natural broods had higher hunting yields per unit time, but similar energy expenditures to those of males rearing smaller natural broods (Masman et al., 1989). In kestrels, differences in hunting yield were due to differences in territory quality.

In an aerial insectivore such as the tree swallow, which does not hold feeding territories, variance among individuals in foraging strategy is probably important. In addition to varying feeding frequency and energy expenditure, provisioning parents have many ways to deal with variation in brood demand. For example, they may vary bolus size or the composition of prey species fed to nestlings (e.g. Wright et al., 1998). These behavioural strategies are not detectable through studies of energy expenditure or feeding frequency.

The absence of a correlation between natural or manipulated brood size and *DEE* is not unusual in birds. Williams and Vézina (Williams and Vézina, 2001) reviewed 20 studies that attempted to correlate *DEE* with brood size. Of these, 14 (70 %) failed to detect a significant correlation. Even when a significant correlation was reported, it was not necessarily consistent between sexes, populations or nestling ages. These previous studies, and the present one, support the hypothesis that adults adjust their brood size to their own feeding capacity (Masman et al., 1989). This may allow all adults within a population to work at similar levels of energy expenditure irrespective of the number of nestlings (Drent and Daan, 1980).

Parental effort: DEE and brood mass

Despite the absence of a correlation between parental *DEE* and brood size, among individuals rearing the same-sized broods, in one of two years *DEE* was related to brood mass. This suggests that there are potential reproductive benefits of a high *DEE*. A positive relationship between *DEE* and brood mass can be interpreted in two ways: (i) parents adjusted their *DEE* to the food requirements of their nestlings, with heavier nestlings requiring more food (e.g. Dykstra and Karasov, 1993) or (ii) parental effort, as reflected by *DEE*, determined nestling growth rates and mass. Moreno et al. (Moreno et al., 1997) argued that, as parental *DEE* rarely responds to changes in brood demand (e.g. Williams and Vézina, 2000), levels of activity are probably constrained by either a parental time/activity budget or a physiological limit to energy expenditure (Drent and Daan, 1980). Consequently, they argued that it is more likely that it is the adult's capacity to attain a high *DEE* that determines a nestling's growth rate and not the reverse (Moreno et al., 1997).

Why we detected a significant correlation between *DEE* and brood mass in only one of the two years is not clear, although it may be due in part to inter-annual variation in abiotic factors (e.g. temperature; see below). Since the data were collected in only two years, it is not possible to assess whether a significant correlation in one year was a statistical artefact. However, given the similar correlations of Moreno et al. (Moreno et al., 1997), we suggest that statistical significance probably represented biological significance.

Physiological and biochemical correlates of DEE

None of the physiological or biochemical characters that we measured showed a correlation with *DEE*. This is consistent with the results of Meerlo et al. (Meerlo et al., 1997), who failed to detect a correlation between *DEE* and the mass of any organ or tissue in field voles (*Microtus agrestis*). Our results and those of Meerlo et al. (Meerlo et al., 1997) contrast with those from studies of cold-exposed laboratory mice (Konarzewski and Diamond, 1994). When food was provided *ad libitum*, individual mice with a relatively high *DEE* had relatively heavy kidneys and intestines, suggesting that high digestive and excretory capacities were required to attain a high level of energy expenditure. A failure to detect such a relationship in the field may indicate that the *DEE* of both swallows and voles was considerably below the physiological maximum of each species.

There has been considerable debate over the proximate physiological cause of a ceiling on energy expenditure (for a review, see Hammond and Diamond, 1997). In the field, however, environmental constraints (e.g. day length) may take precedence over physiological or biochemical ones. For example, Dykstra and Karasov (Dykstra and Karasov, 1993) showed that the *DEE* of provisioning house wrens (*Troglodytes aedon*) was below that measured in the laboratory under conditions of cold and exercise. As a consequence, they argued that provisioning effort in birds is unlikely to be constrained physiologically. Recent work on female great tits (*Parus major*) also supports the existence of ecological constraints on *DEE* (Tinbergen and Verhulst, 2000). Provisioning females reduced their *DEE* in response to an experimental reduction in brood size, but showed no increase with experimental enlargements. This suggested the existence of an energetic ceiling set at the level of an individual's unmanipulated brood size. The energetic ceiling varied between years, demonstrating that it was set by ecological rather than physiological factors (Tinbergen and Verhulst, 2000).

In the present study, the mean *DEE* was greater in 1997 than in 1996. The reasons for this difference are unknown, but it may have been related to environmental temperature. The maximum temperature on the date of capture was higher in 1996 (25.1±3.8 °C) than in 1997 (22.5±3.5 °C; t=2.36, d.f.=45, P<0.05). There was no inter-annual difference in nestling

growth rate, suggesting that the lower *DEE* in 1996 than in 1997 may have been due to reduced thermoregulatory costs (of either the adults or nestlings). In addition to a low *DEE*, adult tree swallows in 1996 had, on average, smaller pectoral muscles and kidneys (females) and livers (males) than in 1997 (Table 2). A causal relationship between inter-annual differences in *DEE* and body composition seems unlikely, however, because there was no correlation between residual *DEE* and the residuals of the mass of any organ. The ecological reasons underlying inter-annual variation in body composition remain unknown (Burness et al., 1998).

Concluding remarks

Energy allocation by parents to offspring is predicted to increase with increasing brood size. Despite this, we could not detect a correlation between parental *DEE* and natural brood size. We hypothesize that more energetically efficient foragers lay larger clutches. This supports a previous suggestion in the literature that clutch size is adjusted to the amount of food that can be delivered to nestlings for the same parental energy expenditure (Masman et al., 1989).

Among individuals rearing the same-sized broods, in one of two years there was a positive correlation between parental *DEE* and brood mass. This suggests that there were potential seasonal reproductive benefits of a high *DEE*. Because there was no correlation between *DEE* and body composition, we hypothesize that individuals were working below their physiological maximum. The absence of a correlation means, however, that individuals with relatively high *DEE*s were unlikely to have paid a penalty in terms of an elevation in resting metabolic rate.

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