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

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

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$_2$
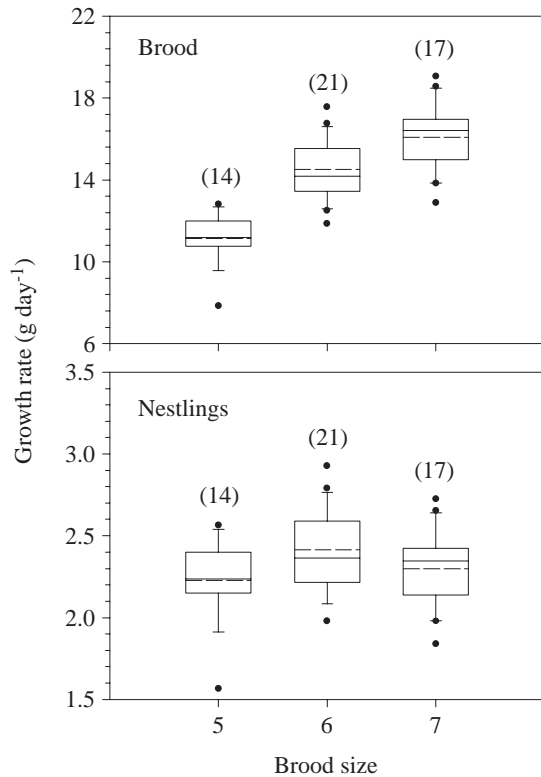
Fig. 1. Box plots of growth rates between days 4 and 8 of entire tree
t

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	4725.6(18.13)710(0)-59.3				

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: $P>0.90$)

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

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*

Masman, D., Dijkstra, C., Daan, S. and Bult, A. (1989). Energetic limitation of avian parental effort: field experiments in the kestrel (*Falco tinnunculus*). *J. Evol. Biol.* **2**, 435–455.

Meerlo, P., Bolle, L., Visser, G. H., Masman, D. and Daan, S. (1997). Basal metabolic rate in relation to body composition and daily energy expenditure in the field vole, *Microtus agrestis*. *Physiol. Zool.* **70**, 362–369.

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

Moreno, J., Potti, J. and Merino, S. (1997). Parental energy expenditure and offspring size in the pied flycatcher *Ficedula hypoleuca*. *Oikos* **79**, 559–567.

Nagy, K. A. (1983). *The Doubly Labeled Water ($^3HH^{18}O$) Method: a Guide to its Use.* UCLA Publication no. 12-1217. Los Angeles, CA: University of California.

Perrins, C. M. and Moss, D. (1975). Reproductive rates in the great tit. *J. Anim. Ecol.* **45**, 695–706.

Piersma, T. and Lindström, Å. (1997). Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol. Evol.* **12**, 134–138.

Potti, J., Moreno, J. and Merino, S. (1999). Repeatability of parental effort in male and female pied flycatchers as measured with doubly labeled water. *Can. J. Zool.* **77**, 174–179.

Ricklefs, R. E., Konarzewski, M. and Daan, S. (1996). The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *Am. Nat.* **147**, 1047–1071.

Robertson, R. J., Strutchbury, B. J. and Cohen, R. R. (1992). Tree Swallow (*Tachycineta bicolor*). In *The Birds of North America*, No. 11 (ed. A. Poole, P. Stettenheim and F. Gill). Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists' Union.

Royama, T. (1966). Factors governing feeding rate, food requirement and brood size of nestling great tits *Parus major*. *Ibis* **108**, 313–347.

Speakman, J. R. (1997). *Doubly-Labelled Water: Theory and Practice*. London: Chapman & Hall.

Speakman, J. R. (2000). The cost of living: field metabolic rates of small mammals. *Adv. Ecol. Res.* **30**, 178–297.

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

Tinbergen, J. M. and Verhulst, S. (2000). A fixed energetic ceiling to parental effort in the great tit? *J. Anim. Ecol.* **69**, 323–334.

Verhulst, S. (1995). *Reproductive Decisions in Great Tits: an Optimality Approach*. PhD thesis, University of Groningen, Haren, The Netherlands.

Weathers, W. W. and Sullivan, K. A. (1989). Juvenile foraging proficiency, parental effort and avian reproductive success. *Ecol. Monogr.* **59**, 223–246.

Williams, J. B. (1988). Field metabolism of tree swallows during the breeding season. *Auk* **105**, 706–714.

Williams, J. B. and Nagy, K. A. (1984). Validation of the doubly labeled water technique for measuring energy metabolism in savannah sparrows. *Physiol. Zool.* **57**, 325–328.

Williams, T. D. and Vézina, F. (2001). Reproductive energy expenditure, intraspecific variation and fitness. *Current Orn.* **16** (in press).

Wright, J., Both, C., Cotton, P. A. and Bryant, D. (1998). Quality vs. quantity: energetics and nutritional trade-offs in parental provisioning strategies. *J. Anim. Ecol.* **67**, 620–634.