DAILY ENERGY EXPENDITURE AND WATER-TURNOVER RATE OF ADULT EUROPEAN STARLINGS (*STURNUS VULGARIS*) DURING THE NESTING CYCLE

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ABSTRACT.—We measured rates of carbon-dioxide production and water turnover in adult starlings by the doubly-labeled water technique. CO_2 production of females was 4.23 $cm^3 \cdot g^{-1} \cdot h^{-1}$ during the incubation period, 4.86 $cm^3 \cdot g^{-1} \cdot h^{-1}$ during the early part of the nestling period, and 6.86 $cm^3 \cdot g^{-1} \cdot h^{-1}$ during the middle of the nestling period, the time of greatest food requirement by the brood. During the last period, the rate of CO_2 production by males was 5.50 $cm^3 \cdot g^{-1} \cdot h^{-1}$. CO_2 production was independent of brood size (3–7, approximately a two-fold range of brood mass) during both the early and middle parts of the nestling period. Water-turnover rates paralleled CO_2 production over the nesting cycle, the average values for each sample varying between 0.048 and 0.070 per h. Within each of the samples, water-turnover rates were independent of CO_2 production but appeared to be influenced by weather conditions. During the middle of the nestling period, water-turnover rates were higher on 2 cold, rainy days than on 2 milder days. The maximum daily energy expenditure for females was about four times predicted BMR, similar to values reported in other studies.
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DAILY energy expenditure (DEE) of adult birds has been the subject of many recent studies (Walsberg 1977, 1978, 1983; Mugaas and King 1981). A few investigators have attempted to relate DEE to variations in the time and energy demands of activities during the nesting cycle and to conditions of the environment. For example, Drent and Daan (1980) concluded that the maximum daily work capacity of birds during the nesting cycle is about four times the basal metabolic rate. Some investigators, using time and energy budget methods, have suggested that there is an increase in DEE between the incubation and nestling periods (Walsberg 1977, Mugaas and King 1981); others have found none (Custer 1974, Withers 1977, Holmes et al. 1979, Ettinger and King 1980). Part of this discrepancy may arise from uncertainty over factors converting activity to energy expenditure.

Although time budgets may be used to obtain a rough approximation of DEE, they may not be sufficiently sensitive to small variations in climatic conditions or behavior to reveal variations in DEE with respect to the stage of the nesting cycle, to weather, or to individual differences within a population. Time budgets are difficult to estimate accurately and must be subdivided into coarse categories of activity. As a result, time-energy budgets may not reveal subtle variations in the power requirements of particular activities. Where time-budget estimates of DEE of free-living birds have been compared with estimates obtained by the doubly-labeled water (DLW) technique, correlations between the pairs of measurements have been poor (Utter 1971, Williams and Nagy 1984a). The DLW technique estimates DEE by recording changes in the proportions of injected water molecules labeled with isotopes of both hydrogen and oxygen (Lifson and McClintock 1966; Nagy 1975, 1980). It has been applied to the Purple Martin (*Progne subis;* Utter 1971, Utter and LeFebvre 1973), the Northern Mockingbird (*Mimus polyglottos;* Utter 1971), and the Common House-Martin (*Delichon urbica;* Hails and Bryant 1979), among passerines. Of these workers, only Hails and Bryant (1979) obtained enough measurements to relate DEE to breeding variables. They found that males, but not females, increased DEE in direct relation to the biomass of the brood.

The purpose of this study was to investigate the relationship between DEE, measured by the DLW technique, and the stage of the nesting cycle in the European Starling (*Sturnus vulgar-*

is). We hyopthesized that the power requirements of breeding adults would increase from the incubation period through the nestling period as the daily energy requirements of the nestlings increased. We manipulated the sizes of broods to determine whether or not DEE responded to altered energy demands at a particular stage of the nesting cycle. We also calculated water-turnover rate of adult starlings.

METHODS

The study was conducted at the Stroud Water Research Center of the Academy of Natural Sciences of Philadelphia, near Avondale, in southeastern Pennsylvania. All work was conducted with first broods during May 1982. We obtained DEE measurements during the incubation stage on 2–5 May, during the early nestling-rearing period (brooding period, nestling age 1–3 days) on May 9–11, and during the middle nestling-rearing period (ages 8–14 days) on May 20–23. The last corresponds to the age of maximum food requirement of nestlings (Westerterp et al. 1982, L. Clark and R. E. Ricklefs unpubl. data).

To determine the time required for isotopic water to equilibrate with body water, we injected two adults intramuscularly with 0.4 cm^3 of tritiated water (0.3 mCi/cm^3) and drew blood samples periodically. From one individual, blood was drawn after 5, 16, 30, 60, and 90 min. The maximum number of counts per minute of tritium was obtained at 30 min, and the value at 16 min was 96% of the maximum. From the other individual, blood was drawn 10, 30, and 60 min after injection. The maximum number of counts was recorded at 60 min, but the counts at 10 and 30 min were 89 and 98% of the maximum. We concluded that 1 h was an adequate period for equilibration of injected labelled water.

To capture birds in the field, we fitted nest boxes with wire traps placed over the entrances and operated manually from a distance. Most birds were caught between 1000 and 1800. They were injected with 0.4 cm^3 tritiated water (0.3 mCi/cm^3) mixed with oxygen-18 enriched water (98 atom%) either intraperitoneally (IP) (nestling period) or intramuscularly (IM) (incubation and brooding periods). In Chukars (*Alectoris chukar*), Degen et al. (1981) found no difference between equilibrium times or levels of isotopes injected IP and IM. In our study, levels of isotopes recovered from blood drawn 1 h after injection also did not differ significantly ($P > 0.05$) between IP and IM injections. Each injected bird was held in an open mesh bag for approximately 1 h. An initial blood sample of 0.20 cm^3 was then obtained by puncturing the brachial vein and drawing blood into heparinized capillary tubes. The tubes were flame-sealed and stored at 4°C until further analysis. Birds were weighed with Pesola spring balances (0.1-g accuracy) to 1 g and fitted with aluminum Fish and Wildlife Service leg bands. Most injected birds were retrapped, after periods of 23–37 h; these birds were reweighed, and a second blood sample was taken from the opposite wing.

To substantiate that birds resumed their normal activities after injection, we observed many nests after adults were released, and in most cases birds returned to incubate eggs or feed nestlings within 1–2 h. We weighed and measured nestlings at the beginning and end of the trial, and, by comparing weight increments of nestlings in experimental and undisturbed nests, we determined that parents were feeding the nestlings normally. We also made spot-checks of activity the next day to be sure both parents were feeding the young. Some broods (normally 5 chicks) were increased or decreased by two chicks several days before the DEE measurements in order to examine the relationship between energy requirement and brood size.

We micro-distilled (Wood et al. 1975) the blood samples under vacuum and measured tritium activity of 10-μl samples (Beckman LS 230 liquid scintillation counter) in 10 cm^3 of a toluene-Triton X 100-POP scintillation cocktail. Each sample was counted three times to 0.7% error; then the counts were averaged. We measured oxygen-18 content by means of cyclotron-generated proton activation of O-18 to fluorine-18 and subsequent counting of the gamma-emitting F-18 with a Packard Gamma-Rotomatic counting system (Wood et al. 1975). This procedure was carried out by K. Nagy at the Laboratory of Biomedical and Environmental Sciences, UCLA.

CALCULATIONS

All calculations were based on levels of O-18 and tritium with backgrounds subtracted. The fractional turnover rate of tritium isotope (k_W, units are per h, i.e. cm^3 turnover per cm^3 water per hour) was calculated by the expression

$$k_W = \frac{1}{t} \ln\left(\frac{T_i}{T_f}\right) \quad (1)$$

(Lifson and McClintock 1966), where T_i is the initial level of tritium in the blood, T_f the final level of tritium, and t the length of the period of measurement (h). When total body water does not change over the period of measurement, k_W is equal to the fractional turnover rate of body water (Nagy and Costa 1980).

The rate of carbon-dioxide production can be estimated by the equation

$$r_{CO_2} = \frac{N}{2}(k_O - k_H) \quad (2)$$

TABLE 1. Body masses of birds used to measure CO_2 production.

Sample period	Sex	n	Mass (g)	SD (g)	CV (%)
Incubation	Female	4	85.0	5.4	6.3
Early nestling	Female	7	78.7	2.7	3.4
Middle nestling	Female	7	74.1	5.2	7.0
Middle nestling	Male	4	76.9	2.8	3.6

(Lifson and McClintock 1966), where N is total body water (mmoles), and k_O and k_H are the fractional turnover rates of oxygen and hydrogen isotopes in body water, estimated by equations analogous to those for water turnover, i.e. $k_O = (\ln O_i - \ln O_f)/t$ and $k_H = (\ln H_i - \ln H_f)/t$. If t is measured in days, r_{CO_2} is expressed as mmoles CO_2/day. To convert equation (2) to cm³ of CO_2 and g of water, R_{CO_2} must be multiplied by 22.4 cm³/mmole for CO_2 and 55.556 mmole/g for water, i.e., 1,244.46. The resulting equation is

$$CO_2(cm^3/h) = \frac{622.23W}{t} \cdot(\ln O_i - \ln O_f - \ln H_i + \ln H_f), \quad (3)$$

where W is total body water (g) and t is time (h).

Equation (3) is applicable only when total body water remains constant during the course of the trial. Several correction factors have been applied when total body water changes (Lifson and McClintock 1966, Nagy 1980). These factors are based on estimating the harmonic mean of total body water under assumptions of either linear or exponential change. We have calculated that for differences in initial and final body water of up to 10%, these correction factors are within 0.2% of the simple arithmetic mean of the initial and final body water, i.e. $W = (W_i + W_f)/2$, which we use here. Also, we can express calculated rates of CO_2 production on a mass-specific basis by dividing equation (3) by the arithmetic average of the initial and final masses of individuals, i.e. $M = (M_i + M_f)/2$. The ratio $(W_i + W_f)/(M_i + M_f)$ is very close to the average water fraction of the individual $WF = [(W_i/M_i) + (W_f/M_f)]/2$, which may be substituted into equation (3) to obtain the expression used to calculate CO_2 production in this study

$$CO_2(cm^3 \cdot g^{-1} \cdot h^{-1}) = \frac{622.23WF}{t} \cdot(\ln O_i - \ln O_f - \ln H_i + \ln H_f). \quad (4)$$

The water fraction was estimated in 29 adult specimens air-dried to constant mass at either 50–60°C or 70°C. Seven adults, which were collected just before roosting on 14 March 1982 at 2000 and which were not sexed, weighed 85.1 g (3.8 SD). Their water fractions averaged 0.646 (0.015 SD, 2.26% CV) and were not correlated with wet mass. The water contents of 22 adults, mostly females collected during the nesting cycle during 1970 and 1971, averaged 0.644 (0.012 SD, 1.82% CV). In this study, we used a water fraction (WF) of 0.64, which has a coefficient of variation of about 2%.

Errors in the estimate of CO_2 production can be made in a number of ways. Repeated pipetting of water distilled from one sample of blood into scintillation vials revealed coefficients of variation in tritium counts consistently less than 1%. The logarithms of repeated measurements of ^{18}O and ^{3}H on the same samples of blood had standard deviations of 0.005–0.018, amounting to a probable error standard deviation of the four values of ln O and ln H together of about 2%. The SD of WF was also on the order of 2%. Hence, the total variation in DEE due to measurement error alone is probably on the order of 5% SD. Validation studies on birds indicate that DLW estimates are ±10% of gravimetric measurements of CO_2 production (Williams and Nagy 1984b).

Because our measurement intervals varied between 23 and 37 h, we estimated DEE by calculating the intercept at 24 h of the regression of total CO_2 produced against interval. All intervals included a full nighttime period. Therefore, variations in the measurement interval represent variations in hours of daytime activity, and the slope of the regression estimates the daytime rate of CO_2 production. Other calculations are described in the results and discussion sections. All calculations were made with the procedures of the Statistical Analysis System (SAS, Helwig and Council 1979). We assumed a respiratory quotient (R.Q.) of 0.75, representing a diet mostly of protein (Schmidt-Nielsen 1975: 211), and therefore used a conversion factor of 26.8 J/cm³CO_2 to convert CO_2 production to energy expenditure.

RESULTS

Carbon dioxide production.—The body mass of females decreased from 85 g during the incubation period to 74 g during the middle of the nestling period (Table 1). Males captured during the nestling period were about 3 g heavier than females. Over the sample as a whole, the absolute value of the percentage of change in body mass during the DLW measurement interval, calculated as percentage of change = $200|M_i - M_f|/(M_i + M_f)$, averaged 2.46% (2.03% SD, $n = 20$), hence approximately 2 g.

Total CO_2 production is plotted as a function

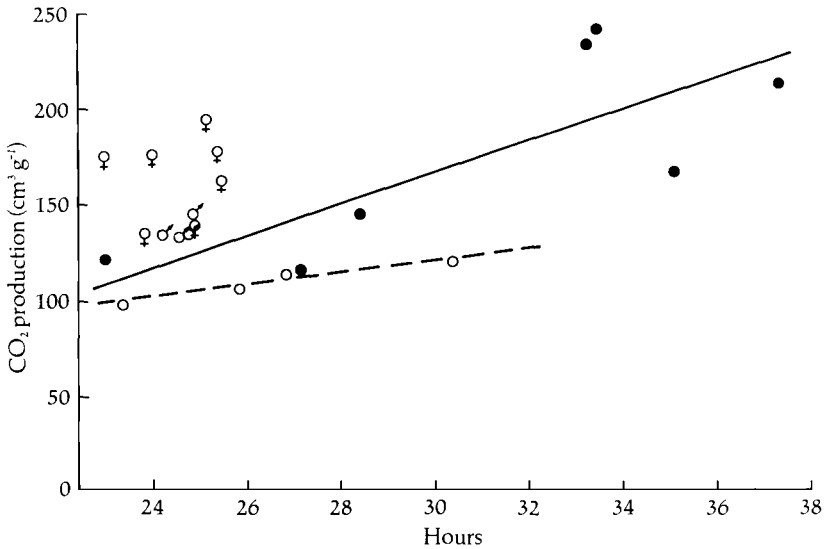

Fig. 1. Relationship between mass-specific CO_2 production and period of measurement. Open circles: incubation period; solid circles: early nestling period; sex symbols: middle nestling period. The lines are the regressions of CO_2 production versus time for the incubation period (dashed line) and early nestling period (solid line).

of the length of the measurement interval in Fig. 1. For the four females tested during the incubation period over intervals ranging from 23 to 30 h, the regression of CO_2 production on time was significant [$F(1,2) = 30.0$, $P = 0.032$, $R^2 = 0.94$], with a slope of 2.96 (0.54 SE) $cm^3 \cdot g^{-1} \cdot h^{-1}$ and an intercept at 24 h of 101.55 (2.04 SE) $cm^3 \cdot g^{-1} \cdot day^{-1}$. Divided by 24 h, the intercept is equivalent to 4.23 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$.

During the brooding period, similar data for seven females had a significant [$F(1,5) = 7.76$, $P = 0.039$, $R^2 = 0.61$] slope of 8.37 (3.01 SE) $cm^3 \cdot g^{-1} \cdot h^{-1}$ and an intercept of 116.70 (25.55 SE) $cm^3 \cdot g^{-1} \cdot day^{-1}$, or 4.86 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$. When data for the incubation and brooding periods were compared in a single analysis of covariance, the interaction term was not significant [$F(1,7) = 0.72$, $P = 0.42$], indicating that the slopes of the regressions, i.e. daytime rates of CO_2 production, did not differ significantly. When we fitted the data by a model with a common slope for the two periods, the regression was significant [$F(1,8) = 10.76$, $P = 0.011$, $R^2 = 0.74$] with slope 7.52 (2.29 SE) $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$ and intercepts of 89.24 $cm^3 \cdot g^{-1} \cdot day^{-1}$ during the incubation period and 122.84 during the brooding period (16.42 SE) [intercepts not significantly different, $F(1,8) = 2.42$, $P = 0.156$].

During the nestling period, intervals between initial and final blood sampling were narrowly confined to the range 23–25.5 h; hence, the data were not suitable for regression analysis. For seven females, the average rate of CO_2 production was 6.89 (0.96 SD) $cm^3 \cdot g^{-1} \cdot h^{-1}$ over an average period of 24.25 (0.77 SD) h. For four males, the rate was 5.56 (0.17 SD) $cm^3 \cdot g^{-1} \cdot h^{-1}$ over an average period of 24.63 (0.28 SD) h.

Because the intervals of measurement were not exactly 24 h, we adjusted estimates of CO_2 production by the excess time over 24 h times the rate of CO_2 production during the daylight hours when the excess occurred. To make this adjustment, we estimated the daytime rate of CO_2 production by subtracting the estimated nighttime metabolism from the total and dividing by the length of the daytime period. The standard metabolic rate was estimated from the equation of Lasiewski and Dawson (1967) for a passerine of 74-g mass (1.723 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$). This value was multiplied by a factor of 1.27, estimated for roosting and incubation by Mugaas and King (1981). The length of the night period was 9.5 h on 21 May at 40°N (List 1966). From the total CO_2 production for 24.25 h (167 cm^3/g), we subtracted the estimated nighttime production of 20.8 cm^3/g to obtain 146 cm^3/g for 14.75 h of daytime activity, i.e. a rate of 9.9

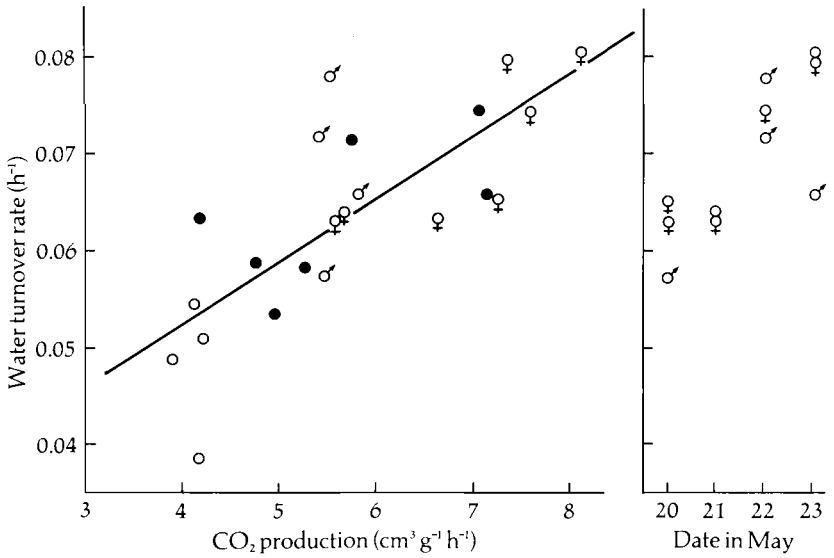

Fig. 2. Relationship between water-turnover rate and mass-specific rate of CO_2 production (left) and date during the middle of the nestling period (right). Symbols as in Fig. 1. The line represents the overall regression of water turnover on CO_2 production.

$cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$. Parallel calculations for a 77-g male (BMR = 1.704) yielded a rate of CO_2 production of 7.7 $cm^3 \cdot g^{-1} \cdot h^{-1}$ for daytime activity. When these values were used to adjust total CO_2 production to exactly 24 h, rates of CO_2 production were 6.9 $cm^3 \cdot g^{-1} \cdot h^{-1}$ for females and 5.5 $cm^3 \cdot g^{-1} \cdot h^{-1}$ for males. These rates correspond to 331 kJ/day for females and 272 kJ/day for males, and they are somewhat higher than the 200–300 kJ/day range for adult starlings rearing 3–5 nestlings reported by Westerterp et al. (1982, no details given).

Effect of brood size.—The sizes of the clutches (5 or 6 eggs) were not manipulated. Of females studied during the early part of the nestling period, 3 had broods of 3, 3 had broods of 5, and 1 had a brood of 7. The average brood mass was 69.8 (25.6 SD) g. There was no relationship between CO_2 production and brood size, brood mass, or change in brood mass.

During the middle of the nestling period, by which time most of the chicks approached fledging mass, the brood sizes varied between 2 and 7, and the average brood mass was 272 (69 SD) g. The rate of CO_2 production varied little among males (see Fig. 1), although brood sizes were 3 (1 individual), 5 (2), and 7 (1). Among females, the two lower values for CO_2 production were obtained from females with broods of 2 and 4 nestlings. The five higher values were obtained from females caring for broods of 3 (2), 5 (2), and 7 (1). Although brood masses varied between 150 and 350 g, there was no relationship between CO_2 production and brood size, brood mass, or change in brood mass.

Water turnover rate.—The hourly rate of water turnover (WTO) did not vary significantly with respect to the length of the measurement period during the incubation and brooding periods, suggesting that the rates of WTO during the day and night did not differ markedly. Average rates of WTO for each of the samples (Table 2) paralleled average rates of CO_2 production. Within the whole sample, WTO (h^{-1}) was significantly [$F(1,20) = 28.03$, $P = 0.0001$, $R^2 = 0.58$] related to the rate of CO_2 production ($cm^3 \cdot$

TABLE 2. Rates of water turnover (WTO) in adult starlings during the nesting cycle.

Period of cycle	Sex	*n*	WTO (h^{-1})	SD (h^{-1})
Incubation	Female	4	0.048	0.007
Early nestling	Female	7	0.064	0.008
Middle nestling	Female	7	0.070	0.008
Middle nestling	Male	4	0.068	0.009

TABLE 3. Water turnover rates (WTO) of male and female starlings during the middle of the nestling period according to date of trial.

Date of injection	n	WTO (h^{-1})	SD (h^{-1})
20 May	3	0.062	0.004
21 May	2	0.064	0.001
22 May	3	0.075	0.003
23 May	3	0.075	0.008

$g^{-1} \cdot h^{-1}$), with slope 0.0065 (0.0012 SE) g/cm^3 (Fig. 2).

During the middle part of the nestling period, WTO for males and females combined was independent of CO_2 production [$F(1,6) = 3.64$, $P = 0.105$] but did vary with date of injection [$F(3,7) = 5.12$, $P = 0.035$, $R^2 = 0.69$], as is shown in Table 3. Values obtained from birds injected on 22 and 23 May were significantly higher than those obtained from birds injected on 20 and 21 May. Carbon-dioxide production was not related to date in this sample [$F(3,7) = 0.55$, $P = 0.66$].

DISCUSSION

We have shown that the DEE of breeding starling females increased from the incubation period through the middle of the nestling period. Furthermore, males expended less energy during the middle of the nestling period than did females. The daily metabolic rate during the period of maximum DEE was either 4.0 or 4.5 times BMR in females, depending on the allometric equation used to calculate BMR, and 3.2 or 3.7 times BMR in males (Table 4). These values are similar to the levels reported in studies of other species (Drent and Daan 1980). Although Mugaas and King (1981) suggested that terrestrial foragers might have lower DEEs than do aerial feeders, our estimates for the starling, which are comparable with those for the Common House-Martin and exceed those for the Purple Martin, would seem to contradict this hypothesis. Furthermore, Walsberg (1983) could find no significant difference between the regressions of DEE upon adult mass for aerial and terrestrial foragers. Compared with Walsberg's (1983) regression equation based on 42 species, DEEs of breeding starlings were consistently higher, by a factor of 1.20 to 1.86 (Table 4).

The increase in DEE during the nesting cycle is consistent with a common perception of the changes in energy demands of reproduction. Brood mass, and presumably the food requirements of the nestlings (e.g. Westerterp et al. 1982), nearly quadrupled between the early and middle stages of the nestling period, during which time most of the postnatal increase in mass is accomplished. During the middle of the nestling period, the CO_2 production of the females was about 12 l/day. The total CO_2 production of a brood of 5 10-day-old nestlings at prevailing ambient temperatures is about 15 l/day (L. Clark unpubl. data). If the female provided half the food needed by the brood, this would amount to about 60% of her total DEE, and perhaps somewhat more if nestling requirements for growth were included.

The lower DEE of males during the nestling period is consistent with the observation that males deliver only about 75% as many meals to the nestlings, especially in small broods, as do females (E. H. Dunn and R. E. Ricklefs unpubl. data, L. Clark unpubl. data), but the difference in metabolic rate is seemingly too large to be accounted for by feeding rate alone. The DEE

TABLE 4. Rate of CO_2 production during different stages of the nesting cycle.

Period	Sex	Mass (g)	CO_2 production ($cm^3 \cdot g^{-1} \cdot h^{-1}$)	Multiple of BMR[a]		Multiple of estimated DEE[b]
				L&D	A&P	
Incubation	Female	85	4.23	2.55	2.89	1.20
Early nestling	Female	79	4.86	2.87	3.25	1.34
Middle nestling	Female	75	6.86	4.00	4.52	1.86
Middle nestling	Male	77	5.50	3.23	3.65	1.51

[a] BMR calculated either by the equation of Lasiewski and Dawson (1967) for passerines or of Aschoff and Pohl (1970) for passerines during the inactivity phase of the daily cycle.

[b] DEE calculated by the equation of Walsberg (1983).

of males is about midway between that of females during the incubation and middle of the nestling periods, whereas we would have expected males to be positioned about ¾ of the way between the two.

Bryant and Westerterp (1980) found that the DEE of four incubating female Common House-Martins was 6.20 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$, or approximately 2.7 times the estimated BMR based on Aschoff and Pohl's (1970) equation. During the chick-rearing period, the average DEE of 56 males and females was 8.17 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$ (3.59 times BMR), or 32% greater than during the incubation period. Starlings had an increase in DEE of 62% in this study, but it is not clear how comparable the two studies are. In the starling, DEE was lower during the early nestling period than during the middle nestling period, and lower for males than for females during the middle of the nestling period. Bryant and Westerterp averaged their data during the nestling period. Hails and Bryant (1979) also reported that the DEE of male Common House-Martins increased in direct relation to the size and mass of the brood. No such trend was evident in this study, but Hails and Bryant's data included males brooding small chicks, and therefore the correlation of DEE and brood mass may have reflected the amount of time spent brooding rather than energy expended for foraging.

We calculated nighttime CO_2 production from allometric equations for BMR and calculated daytime production by taking the difference between the nighttime production and the total. In four samples of adults, estimated nighttime production varied between 20 and 21 $cm^3\ CO_2/g$, which was about one-sixth of the total during the incubation period and one-eighth of the total for females during the middle of the nestling period. Estimated daytime rates of production varied from 5.7 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$ during the incubation period to 6.7 during the early nestling period and 7.7 (males) and 9.9 (females) during the middle of the nestling period. These values are consistent with the estimate of daytime CO_2 production based on the common slope of the relationship between CO_2 production and measurement interval during the incubation and brooding periods (7.5).

If the CO_2 production of a bird foraging on the ground is about 2 times BMR (Mugaas and King 1981), or 3.44 $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$, and CO_2 production during flight is approximately 16 $cm^3 \cdot g^{-1} \cdot h^{-1}$ (Torre-Bueno and LaRochelle 1978), the estimated daytime CO_2 production of females during the nestling period (9.9 $cm^3 \cdot g^{-1} \cdot h^{-1}$) would suggest that they spend almost 50% of their time in flight. Observations of adults in our colony indicate that the proportion of the day spent in flight is approximately 1.8 h/day or about 12% of the daylight period (L. Clark unpubl. data), even during the middle of the nestling period. Furthermore, Drent and Daan (1980) quote work of J. M. Tinbergen revealing a maximum of about 3.5 h in flight per day by breeding starlings. Either our estimate of daytime energy expenditure is too high or estimates of the power requirements of ground foraging are too low.

Water-turnover rates of breeding starlings varied from 0.048/h during the incubation period to 0.070/h for females during the middle of the nestling period. These are equivalent to daily WTO rates of 116–168% or (× 0.64) about 74–108% of body mass per day. Torre-Bueno (1978) determined that starlings flying in a wind tunnel at 15°C lost about 1.5% of their mass per hour, i.e., a WTO rate of about 0.0234/h. Because respiratory water loss during flight probably exceeds that during any other activity, the comparison between the flight data and our observations suggests that starlings obtain an excess of water in their diet. We do observe them drinking frequently in the field.

We calculated the amount of water obtained from food by assuming that insects contain 67% water, 18% protein, 3% fat, and 2% carbohydrate (Morton 1973). Hence, 1 g of insect food could provide 0.67 g H_2O as preformed water and about 0.13 g as oxidative water if fully metabolized (Schmidt-Nielsen 1975: 417). If standard energy equivalents (Ricklefs 1974) and 67% assimilation of energy (Gibb 1957, Schartz and Zimmerman 1971) are assumed, 1 g of insect food would also provide about 3.2 kJ, which is equivalent to the production of 120 $cm^3\ CO_2$. These values yield a ratio of 6.3 g H_2O/(1 CO_2). According to our measurements, females during the middle of the nestling period turn over about 6.5 g H_2O/(1 CO_2), which agrees well with our estimate.

During the nestling period, water-turnover rates were lower for birds injected on 20 and 21 May than on 22 and 23 May. Weather records for Philadelphia (U.S. Dept. of Commerce

TABLE 5. Weather summary for Philadelphia International Airport, 20–25 May 1982.

Date	Temperature (°F) Max	Min	Dep[a]	Precipitation (inches)	Average wind (mph)	Relative humidity (%)[b]
20 May	82	64	+9	0.60	9.8	53
21 May	71	56	−1	trace	8.5	68
22 May	58	53	−9	0.09	11.8	86
23 May	56	52	−11	0.18	12.9	83
24 May	58	51	−11	trace	7.5	87

[a] Departure of the average for that day from the long-term average for the date.
[b] Lowest recorded reading for the day.

1982), which are representative of the conditions at the starling colony, indicate that 20 and 21 May were warmer and had lower relative humidity than 22 and 23 May (Table 5). The higher water-turnover rates observed during the colder and more humid period were completely unexpected. Ingestion of rainwater, perhaps with food, or exchange of rainwater across wetted skin, might cause this pattern.

CONCLUSIONS

Our use of doubly-labeled water to estimate daily energy and water-turnover rates has revealed patterns consistent with expectations based on the energy demands of different stages of the breeding cycle. We could not, however, demonstrate any relationship between brood size or mass and DEE within stages of the nestling period, but our samples were small and variation in brood mass within each stage was less than half as great as variation in average brood mass between the early and middle part of the nestling period. The nearly four-fold increase in brood mass from the early part of the nestling period to the middle part was matched by only a 62% increase in DEE of parents, some of which might be accounted for by cold and rainy weather during the latter measurements. The situation is further complicated by the fact that two parents care for the young and, unless one can measure the DEE of both parents or monitor the amount of food provided by each to the young, variation in DEE might, in part, be compensated for by the DEE of the other parent. We were able to measure DEE for two pairs of birds during the middle of the nestling period. The first pair had a brood of seven 8–9-day-old nestlings, which lost a total of 27 g during the 24-h measurement period (22–23 May). The second pair had a brood of five 11–12-day-old nestlings, which gained a total of 11 g. The DEEs of the first pair were 5.48 (male) and 7.26 (female) $cm^3\ CO_2 \cdot g^{-1} \cdot h^{-1}$, and those of the second were 5.53 and 7.59 $cm^3 \cdot g^{-1} \cdot h^{-1}$.

Although Hails and Bryant (1979) were able to detect a weak relationship between brood mass and DEE in male Common House-Martins, it seems unlikely that the doubly-labeled water technique can characterize the relationship between DEE and offspring production adequately to shed light on questions concerning the optimization of reproductive effort in birds. Besides the inherent variability of the technique due to measurement bias and error, the variable effects of the initial trauma, the variation in the allocation of effort between the sexes, and the influence of physical conditions in the environment, it is not clear how measurements of energy expenditure will be used to characterize the relationship between adult risk and realized fecundity, the understanding of which is fundamental to resolving the evolutionary optimization of reproductive effort (Gadgil and Bossert 1970, Stearns 1976, Ricklefs 1983). It seems unrealistic to hope that one could either determine the relationship between DEE and fecundity or translate DEE into adult risk of death.

A goal of studies of DEE is to measure the relative allocation of gathered energy to the demands of reproduction, self-maintenance, and foraging. Partitioning of DEE could be accomplished in multiple regression analyses of variation in DEE with respect to variation in the individual's allocation of time among activities, but the large samples required, owing to the errors in the method, will make suitable studies prohibitively expensive except in the most carefully chosen cases. One may, nonetheless, describe the partitioning of time and energy allocation by combining estimates of DEE obtained by DLW techniques with estimates of the power requirements of various activities measured in other studies (Mugaas and King 1981, Walsberg 1983). In addition, application of the DLW technique may be improved in a number of ways to make it more useful as a field method. One of these is to eliminate the equilibrium period and initial blood sample, as explained in the Appendix. This not only re-

duces the number of isotope analyses, thereby making the technique more economical, but it also eliminates some of the trauma to the experimental subjects and may reduce a source of variation and bias in estimates of DEE.

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APPENDIX

Equation (4) may be rearranged to

$$CO_2(cm^3 \cdot g^{-1} \cdot h^{-1}) = \frac{622.23WF}{t}\left(\ln \frac{O_i}{H_i} - \ln \frac{O_f}{H_f}\right). \quad (5)$$

Hence, if one can estimate $\ln(O_i/H_i)$ independently, then the initial blood sample can be eliminated, and subjects need not be held for a prolonged and perhaps traumatic equilibration period (see also Nagy et al. 1984). Because the initial ratio of the isotopes in the equilibrated blood should be determined primarily by their ratio in the injected water, initial ratios should be sufficiently uniform to be estimated from trials on subjects not released for DEE measurements. The same subjects can be used to determine the average water fraction. In the present study, we injected birds with two lots of isotopes. Initial blood samples from birds injected with the first lot had ratios of H_i/O_i that averaged 46,668 (1,082 SD, 361 SE, 2.32% CV, $n = 9$); blood samples from the second group had ratios that averaged 57,609 (1,250 SD, 395 SE, 2.17% CV, $n = 10$). Therefore, the ratio O_i/H_i can be estimated accurately, perhaps adding only 1% to the probable error of the estimate of DEE. We recommend a sample size of 4–9 individuals to reduce the standard error of the estimate to ½ to ⅓ of the standard deviation. The appropriate period (t) to be used in equation (5) is the total period from injection to final capture minus the equilibrium period of subjects used to estimate the initial isotope ratio. It should be noted that estimates of water-turnover rates require measurement of the absolute value of H_i, which is subject to much greater error than the measurement of H_i/O_i (see Nagy et al. in press). Hence, this abbreviated technique should not be used in studies where accurate water-flux measurements are important.