State-dependent behaviour in breeding barn swallows (*Hirundo rustica*): consequences for reproductive effort

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Life-history theory offers an explanation for the intraspecific variation in reproductive effort; increased levels of current reproductive success, for example, may trade off against residual reproductive value. Even where such trade-offs have been demonstrated, however, much variation in effort remains unexplained and the underlying causes are usually obscure. We examined body state, i.e. energy reserves, as a factor, which could moderate reproductive effort. Specifically, overnight heating and cooling treatments were used to adjust dawn energy reserves in female swallows attending their nests without impinging on the opportunities for foraging. Changes in reproductive effort were measured as 'daytime energy expenditure' (doubly labelled water technique) and the 'number of feeding visits' during brood rearing, which both relate positively to current reproductive success. Our experimental treatments and responses were then compared using the common currency of energy. In response to positive and negative state manipulations, female swallows increased and decreased, respectively, their daytime energy expenditure (and number of feeding visits). These responses to experimental manipulation of state provided evidence of a direct link between the energy expenditure, life history and behaviour, which has hitherto proved elusive. They allow that energy supply and expenditure play a regulatory role in reproductive effort, and indicate that units of energy expenditure probably carry fitness costs and benefits, which are context dependent.

Keywords: body state; energy; trade-offs; reproduction; life-history theory

1. INTRODUCTION

One of the fundamental issues in life-history theory concerns the allocation of effort to reproduction and survival (Stearns 1992). It is generally thought that energy allocated to reproduction (here called reproductive effort) takes resources that might otherwise have been destined for survival or subsequent reproduction (Williams 1966). Much unexplained variation can be seen in field studies of this trade-off, however, involving a range of possible fitness costs or benefits for a given level of effort (Van Noordwijk *et al.* 1981; Nur 1986). Environmental 'noise', parasite loads or individual quality are often proposed as sources of this variability. In addition, recent dynamic models of resource allocation have shown that an individual's physiological state (also called body state or body condition) may play a role in life-history decisions (McNamara & Houston 1992; Houston & McNamara 2000) and thereby affect reproductive effort. Yet, to date, only a few studies have provided evidence that state-dependent behaviour indeed occurs (Bouchon 1991; Damsgard *et al.* 1997; Olsson 1997; Godfrey & Bryant 2000) and the bulk of this work is neither experimental, nor related to reproduction. Furthermore, the quantitative effects of manipulations of specific state variables are invariably unknown (McNamara & Houston 1996). Accordingly, in this study we manipulated the dawn energy reserves of adult female barn swallows (*Hirundo rustica*) during chick rearing. We did this via overnight

heating and chilling treatments, which had known effects on the physiological state; warming treatments conferring a 'positive' energetic state at dawn relative to controls and chilling treatments a 'negative state'. Induced changes in reproductive effort were measured using the doubly labelled water (DLW) technique (Lifson & McClintock 1966) and were characterized by observations of provisioning rates at the nest.

To develop specific hypotheses concerning responses of swallows to experimental changes in body state, we modified the familiar convex trade-off function relating reproduction to survival (Stearns 1992) so that it dealt with reproductive and somatic allocation of resources (figure 1). We assumed that the relationship between reproductive and somatic allocation did not have to be symmetrical (figure 1*a*); hence, a bias may occur in either direction (figure 1*b* or 1*c*). Under the two treatments in this study, which involved positive and negative manipulations of body state, we predicted different optima depending upon the form of the trade-off function. Accordingly, we expected energetic and related behavioural responses under our contrasting manipulations to comply with one of the hypotheses put forward in table 1. The trade-off between reproduction and somatic investment may be symmetrical, with corresponding consequences for reproductive effort or behaviour (H_i), or it may be biased towards somatic input (H_{ii}), or reproductive output (H_{iii}). Alternatively, if no trade-off of this type exists, then reproductive effort or behaviour would not change in response to body state changes (H_0) (table 1).

We justify our assumption that body state manipulations, which increased the swallow's reserves relative to controls, are favourable (i.e. positive), as follows. It has

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Figure 1. Allocation of energy between reproductive and somatic investment is usually assumed to be symmetrical (a). This need not apply, however, as there may be a bias toward either somatic investment (b) or reproductive investment (c). The area under each curve is constant and represents the amount of energy acquired by an individual under each treatment group. The optimal allocation for an individual changes with each treatment, but there is also a shift in position within treatment groups under the two different bias hypotheses (H_{ii} and H_{iii}). Solid line, natural conditions (control); dotted line, warmed treatment; thin line, chilled treatment; filled circle, optima; R, reproductive investment; S, somatic investment.

been shown (Jones 1987) that female swallows have a 'programmed' mass loss (Freed 1981), which continues throughout incubation and into the early nestling period (nestling day 0–7). After this, body mass tends to stay constant, although rising in response to greater food abundance and favourable weather, in unison with provisioning rates, and vice versa. Therefore, while there may be an optimal mass within this latter period (the time of our observations), it is clear that environmental factors also induce mass changes.

2. MATERIAL AND METHODS

(a) *Study species*

The barn swallow (*H. rustica*) is an aerial insectivore, which breeds in the Northern Hemisphere and, in the case of British swallows, migrates to southern Africa during the winter. It nests commonly in farm buildings, laying clutches of four to five eggs in two successive broods. Nestlings typically fledge at the age of 18–21 days. Our experiments were undertaken near Stirling in central Scotland (56°8' N, 3°54' W) in June–August 1997/1998. They involved swallows rearing broods aged 10–14 days. Broods were not used before 10 days because provisioning rates were consistently lower, or after 14 days to guard against premature fledging.

(b) *Manipulations*

Swallows were caught using mist nets between 17.00 and 19.30 at local farms. Sex, mass and fat-score were recorded, fat-score as a rank (0–5) of visible fat deposits in the interclavicular pit (Krementz & Pendelton 1990; Scott *et al.* 1995); a method used reliably with breeding hirundines (Bryant & Westerterp 1983; Bryan 1996). Breeding pairs were randomly assigned to one of three groups; warmed, chilled and control, to induce differences in body state. Thermostatic energy expenditure was lowered by the warming treatments, leading to relatively high residual energy reserves at dawn. The converse applies after chilling, due to the effect of temperatures below the lower critical temperature ($^{\circ}T_{lc}$), which would have increased catabolism of energy substrates. Once initial blood samples had been taken in the laboratory for DLW studies (see § 2c), our warming and chilling treatments were performed overnight, typically from 20.00 to 03.30. During this time, birds were kept in 4.4 l containers equipped with a perch, allowing a normal posture and space for the wings to be fully spread. Only females underwent body state manipulations (warmed and chilled groups). Warmed females were placed in an incubator (Sanyo) in the dark at 29 °C (i.e. within the thermoneutral zone; Kendeigh *et al.* 1977). Chilled females were placed in an incubator programmed to start at 15 °C for 1 h, to decline to 7 °C over 1 h, and then remain at that temperature for the remainder of the night. All control females and male partners of treated females were placed in an outdoor shelter (average night treatment period across groups was 7.2 ± 0.5 h s.d.) and therefore experienced prevailing ambient temperatures (13.8 ± 2.0 °C s.d.), which were recorded every 15 min using a data logger (Psion) equipped with a thermocouple logging. Although 'control females' and male partners of treated females did not undergo manipulations, they were held overnight to control for any behavioural effects of holding birds as temporary captives.

Birds were released at dawn (between 03.30 and 04.00) to ensure a normal foraging period, after mass and fat-score had been recorded again. This information was not obtained from all birds due to accidental releases (table 2). During the release period (mean time to recapture: 16.4 ± 1.5), pairs were observed (typically between 08.00 and 12.00), using video cameras (Panasonic, model NB-585B) at the nest to obtain 'feeding visit rates' (observations lasted 1.4 ± 0.4 h per nest), and from this visits per hour were calculated. Since feeding rates may change with age, nestling age was added as a covariate in the statistical analysis described below. Visit rates for one pair of birds were lost due to video damage. Approximately 65% of birds were recaptured for DLW measurements and final blood samples taken ca. 24 h (23.6 ± 1.3 h) after the initial blood samples. Mass and fat-score were recorded once again.

Table 1. Hypotheses related to the trade-off functions shown in figure 1.

(Specific predictions of responses to positive and negative state manipulations in female swallows are also shown for the four dependent variables tested in this study. Note that energy expenditure refers to AEE, post manipulation.)

	warmed females	chilled females
H_0 : no trade-off	energy expenditure = controls nest visitation = controls mass/fat-score changes = controls	energy expenditure = controls nest visitation = controls mass/fat-score changes = controls
H_i : symmetrical trade-off	energy expenditure > controls nest visitation > controls mass/fat-score changes > controls	energy expenditure < controls nest visitation < controls mass/fat-score changes < controls
H_{ii} : biased trade-off 1: variable somatic investment	energy expenditure > controls nest visitation = controls mass/fat-score changes > controls	energy expenditure < controls nest visitation = controls mass/fat-score changes < controls
H_{iii} : biased trade-off 2: variable reproductive investment	energy expenditure > controls nest visitation > controls mass/fat-score changes = controls	energy expenditure > controls nest visitation > controls mass/fat-score changes = controls

The energetic costs of overnight treatments were quantified using indirect calorimetry with a subsample of birds, some of which were not drawn from experimental groups ($n = 15$). A VG quadrupole mass spectrometer was used to determine oxygen and carbon dioxide concentrations in an open-flow respirometer. From this we derived estimates of energy expenditure overnight. Details of equipment and methods can be found in Bryant & Furness (1995), where an identical approach was used. Food abundance was measured using a 12.2 m Rothamsted suction trap situated at the University of Stirling (Macauley *et al.* 1988). Daily maximum and minimum temperatures were taken from the Parkhead weather station sited on the University campus, within 15 km of all study sites. In addition, during observation of each pair, local temperature, rainfall, and wind were recorded simultaneously near nest sites. Rainfall was recorded as the proportion of time within each observation period when precipitation occurred.

(c) Doubly labelled water

DLW was used to measure energy expenditure by free-living swallows (Lifson & McIntock 1966; Tatner & Bryant 1989). Birds were given intraperitoneal injections of DLW ($10 \mu\text{l g}^{-1}$ body mass). The injectate was prepared by adding 0.37 g of 99.9 atom per cent excess (APE) deuterium (^2H) to 5 ml of 20 APE oxygen-18 water (H_2^{18}O). Once injected, the birds were kept in a bag for 1 h to allow the isotopes to reach equilibrium with the body water (Bryant & Westerterp 1982). Blood samples were then taken in capillaries (Vitrex) from the femoral vein and immediately flame sealed. Six to ten capillaries, each containing 5 μl of blood, were taken from each bird at the time of 'initial' and 'final' samples. Background isotope levels were obtained from blood samples of non-experimental birds ($n = 2$ (1997), $n = 3$ (1998)). Background levels were found to be 1995.5 ± 7.1 (s.d.) for ^{18}O and 146.0 ± 4.0 (s.d.) for ^2D . Isotope concentrations were determined from duplicate samples by mass spectrometry and a mean value was used in subsequent calculations (Tatner & Bryant 1989; Speakman 1997a). Samples for two birds were lost during processing.

(d) Calculation of energy expenditure

Daily energy expenditure (DEE, kJ day^{-1}) was calculated as described by Tatner & Bryant (1989) and Lifson & McIntock (1966), taking a respiratory quotient of 0.75. The body water pool

was derived for each individual using Bryant & Westerterp's (1982) equation for a closely related species (house martin, *Delichon urbica*) during the breeding season. Overnight metabolic cost was obtained from the respirometry results (Gessaman 1987) and a mean obtained for each treatment group ($R \text{ kJ g}^{-1} \text{ h}^{-1}$). From this the cost of the overnight period was calculated using equation (2.1) (W = mean overnight body mass, g; t represents the hours a bird spent in the incubator). The overnight costs were then used to calculate, by difference from the DEE, the 'active' energy expenditure (AEE) of each individual during the release period (equation (2.2)).

Basal metabolic rates (BMRs) were derived from the passerine equation of Aschoff & Pohl 1970, resting phase, and used to provide a standard for comparisons between individuals (called metabolic intensity, MI, equation (2.3)).

$$\text{Overnight costs (kJ)} = R * W * t, \quad (2.1)$$

$$\text{AEE (kJ)} = \text{DEE (kJ)} - \text{overnight costs (kJ)}, \quad (2.2)$$

$$\text{MI} = \text{overnight costs (kJ)} / \text{BMR}. \quad (2.3)$$

(e) Statistical analysis

Data were collected over two years. Hence, to control for random year effects and non-orthogonality due to different treatments each year (warmed in 1997 and chilled in 1998, controls were available for both years), residual maximum likelihood (REML) analysis was used to explain the variation in energy expenditure, visiting rate, fat-score and body mass data. REML is a linear mixed model able to analyse unbalanced, non-orthogonal data (Patterson & Thompson 1971). A similar approach to this has been used recently by Kruuk *et al.* (1999). Stepwise deletion of fixed variables from the minimal adequate model was performed. The random model controlled for the year in which the treatment was performed and the date of the trial within each year. The significance of fixed variables was estimated using the Wald statistic, which is asymptotically distributed as χ^2 (Genstat 5 Committee 1992). *Post hoc* analysis was undertaken using a ratio of the estimated effects and standard errors of the difference between pairs (distributed as t). Overnight mass change and respirometry results were analysed using ANCOVA (Minitab 1997). Analysis of overnight energy costs was performed upon reciprocal transformed, $1/(x - 1)$, energy expenditure (R) to normalize residuals and to control

Table 2. (a) Results of statistical analysis (REML).

(All significant terms ($p < 0.05$) are in bold. χ^2 statistic, degrees of freedom, and probabilities are shown for each variable in the minimal adequate model. (b) Sample sizes for each treatment group within each analysis performed.)

(a)				(b)		
	χ^2 -statistic	d.f.	p -value		female	male
adult mass changes						
brood size	6.46	1	0.011	warmed	8	5
initial mass	5.52	1	0.019	chilled	5	4
treatment	0.03	2	0.986	control	8	8
sex	0.19	1	0.663			
treatment $\times$ sex	4.11	2	0.128			
residual d.f.		34				
adult fat-score changes						
initial fat-score	9.26	1	0.002	warmed	8	5
treatment	0.75	2	0.688	chilled	5	4
sex	0.16	1	0.690	control	8	8
treatment $\times$ sex	1.31	2	0.519			
residual d.f.		35				
AEE						
treatment	4.52	2	0.104	warmed	8	5
sex	0.46	1	0.497	chilled	5	3
treatment $\times$ sex	19.80	2	< 0.0001	control	8	7
treatment $\times$ visiting rate	7.10	2	0.029			
residual d.f.		25				
visiting rate						
brood size	5.63	1	0.018	warmed	7	7
treatment	6.68	2	0.035	chilled	5	5
sex	2.24	1	0.134	control	15	15
treatment $\times$ sex	32.21	2	< 0.0001			
residual d.f.		46				

homogeneity of variance. Predicted means were back-transformed and converted to MI data (equation (2.3)). Sample sizes for analyses are given in table 1. All residuals were checked for normality and homogeneity of variance. Predicted means $\pm$ standard error from statistical analysis, (REML, ANCOVA) are quoted in the text and the figures.

3. RESULTS

(a) *Overnight energy expenditure*

Body state manipulations affected the overnight energetic costs of birds. Warmed birds had the lowest costs ($MI = 1.1 \times BMR$), and chilled the highest ($MI = 2.6 \times BMR$). Control birds had an intermediate overnight cost of $2.1 \times BMR$. There was a significant difference between treatment groups with respect to energy expenditure (table 2), but there was no effect of treatment on mass loss overnight (table 2).

(b) *Active energy expenditure*

Thirty-six swallows were available for analysis of AEE (21 from 1997 and 15 from 1998). Covariates which have been shown to, or were likely to, affect swallow foraging strategy and parental effort, such as food abundance and daily temperature (Bryant & Turner 1982; Jones 1987), were included in this analysis, but none explained a significant amount of variation. Visiting rate to the nest, however, showed significant interactions with both treat-

ment group ($\chi^2_2 = 7.10$, $p = 0.029$) and sex ($\chi^2_1 = 3.57$, $p = 0.059$). Visiting rate showed a significant decrease in the chilled group, possibly involving a decrease in visiting rate of both members of chilled pairs. A significant increase was not seen in the warmed group. Males tended to show a higher visiting rate than females within this analysis. Although the above interactions explained a significant amount of variation and thus were retained, this was only a subsample (65%) of visiting rate data and a more complete dataset is examined below. The model (figure 2a) shows that imposing a different energy state upon an individual causes an energetic response. Control males and females showed very similar levels of AEE (predicted means: 53.2 ± 3.0 kJ (female), and 51.5 ± 3.2 kJ (male)). Warmed females, however, increased their AEE to 66.1 ± 3.0 kJ. An opposite response was seen in the chilled females, which decreased their AEE after treatment to 28.0 ± 6.8 kJ. *Post hoc* analysis within REML, revealed a significant increase in AEE by warmed females ($t_{25} = 3.09$, $p = 0.003$) and a contrary and significant response in chilled females ($t_{25} = 3.36$, $p = 0.013$). Since a mean overnight cost was used to calculate AEE, sensitivity analysis was performed to validate this method. Two REML analyses were run, one using AEE generated using the lowest control (mean - s.d.) and the highest chilled (mean + s.d.) overnight costs, and another using the highest control and lowest warmed costs. This was an attempt to determine if significant

Figure 2. (a) Predicted means $\pm$ standard error (REML) for males and females showing AEE (i.e. energy expended (kJ) during the release period, 16 ± 1.5 h), for the control and treatment groups. (b) Predicted mean visiting rates $\pm$ standard error (h^{-1}) to nests by males and females of the control and treatment groups. Black bars, warmed; dark grey bars, chilled; light grey bars, control.

treatment–sex interactions occurred at the limits of the group distributions. Both analyses revealed a significant interaction term, and *post hoc* analysis indicated the significant increase of warmed females and decrease in chilled females held. Thus, we assert that, although not ideal, the use of means is acceptable in this case. The AEE of the chilled pair males was significantly higher than controls (57.7 ± 4.6 kJ: $t_{25} = 3.31$, $p = 0.0015$), whereas warmed pair males only showed a trend towards increased energy expenditure (53.0 ± 4.8 kJ: $t_{25} = 1.38$, $p = 0.180$).

(c) Feeding visits to the nest

Fifty-four swallows were involved in the analysis of feeding visits to the nest ($n = 35$ in 1997, $n = 19$ in 1998). Nestling age had no effect on feeding visits ($\chi^2_1 = 0.09$, $p = 0.762$) and thus it was excluded from the model. Weather variables and food abundance also proved non-significant terms, but birds with larger broods tended to visit more often than those with relatively smaller broods. The model (treatment $\times$ sex interaction) indicates similar patterns to those for AEE (figure 2b), with control pairs having similar rates, although males seemed to show a slightly higher visiting rate (female: 11 ± 1 , male: 13 ± 1 visits per hour). Warmed females increased their visits (to $18 \pm 2 \text{ h}^{-1}$), and chilled females decreased their visits (to

$4 \pm 3 \text{ h}^{-1}$) in response to the treatments. The visiting rates of pair males from both warmed and chilled groups were not significantly different from controls (13 ± 2 and $10 \pm 3 \text{ h}^{-1}$, respectively). *Post hoc* tests revealed that the changes in visiting rate seen in warmed and chilled females differed from controls ($t_{46} = 4.75$, $p < 0.0001$, and $t_{46} = 2.19$, $p = 0.033$, respectively).

(d) Symmetry of 'input' and 'output' energy values

The mean energy value of inputs was calculated from the model constructed to explain energy expenditure overnight (ANCOVA): R (kJ) (same variables used as table 2a). The predicted mean overnight cost for control females (17.0 ± 0.7 kJ) was then subtracted from all overnight energy expenditure data, in order to obtain a relative difference for treatment females. The analysis was then repeated using these differences as the dependent variable, and the resultant fitted means were used as input estimates, i.e. an energy saving of 8.0 ± 0.8 kJ for warmed females, and a similar extra cost of 5.4 ± 0.9 kJ for chilled females. Output was calculated in a similar way, using a REML model to explain AEE (kJ) (table 2b) for all data. The mean fitted value for control females was again subtracted from the AEE data and the analysis repeated. This gave an increased energy expenditure amongst warmed females of 13.0 ± 3.0 kJ ($0.5 \times \text{BMR}$), and a decrease of 25.2 ± 6.8 kJ ($0.9 \times \text{BMR}$) for chilled females. *Post hoc* analysis showed no significant difference between the absolute values of these two output expenditures ($t_{24} = 1.64$, $p = 0.114$). Accordingly, we interpret these responses as symmetrical with respect to the control and each other, although the power of this analysis was only 43%.

By contrast, energetic input and output values were not equal. Hence, the response to our manipulations involved apparent overcompensation in relation to the energy that was either made available, or withdrawn from reserves, due to our treatments. The output : input ratios ranged from *ca.* 2, in warmed females, to *ca.* 4.5 in chilled females. This latter figure accounts for *ca.* 30% of the DEE of our swallows.

(e) Mass and fat-score change

Treatment, release period or weather variables had no effect on mass changes in experimental swallows over the trial period (table 2). There was, however, a significant effect of initial mass on mass change showing that heavier birds lost more mass over the trial period. Birds with larger broods showed a significantly greater loss of mass. Fat-score changes were significantly affected by the initial fat-score ($\chi^2_1 = 9.26$, $p = 0.002$). No other variable entered into the model, including treatment, explained any significant variation (table 2). Both the mass and fat-score analyses held a statistical power of greater than 80%, to detect a mean change of 0.5 of a standard deviation of their respective distribution. This is equal to *ca.* 0.4 g in terms of mass.

4. DISCUSSION

Our experiments demonstrated a strong association between body state and energy expenditure during repro-

duction in swallows. Therefore, we rejected H_0 , which predicted no effect of body state on reproductive effort. Our results are consistent with a trade-off biased towards reproductive effort (H_{iii}).

Under normal circumstances an individual can only increase reproductive effort at the cost of future breeding success or survival (Williams 1966; Nur 1984; Partridge & Harvey 1985; Partridge 1992; Henderson & Hart 1993; Jacobsen *et al.* 1995). By manipulating body state (energy reserves at dawn), however, and hence, by implication, the probability of future survival (or breeding opportunities), we induced short-term changes in reproductive effort, measured in two complementary ways. Positive state manipulations induced additional effort, and negative manipulations led to a reduced effort, when compared to a control level. We therefore conclude that somatic energy storage (and associated survival risks) traded off against the work done on provisioning the brood. This result was consistent with hypothesis H_{iii} , whereby body state regulates AEE and behaviour during breeding, however H_i cannot be discounted on this evidence alone.

There were no differences in mass or fat-score changes amongst the three groups over the trial period; thus experimental birds returned to an equivalent mass or fat-score by the end of the period. If energy expenditure was increasing in parallel with visiting rate, and somatic investment did not vary substantially between treatment groups, the results were only consistent with hypothesis H_{iii} , and H_i , where some changes in parental mass and fat-score were predicted, can be discounted. Specifically, following our manipulations of body state, the subsequent response showed a bias towards reproduction, and no impact on mass or fat storage, implying a skewed underlying fitness function relating reproduction to somatic investment (figure 1c). Therefore, reproductive effort is the parameter most likely to be sacrificed or boosted by barn swallows when their body state is perturbed, with energy being a currency, which is integral to both the perturbation and the response.

The model outputs of energy expenditure and visiting rates were very similar (figure 2a,b), and visiting rate played a significant role in explaining the variation seen in our energy expenditure data. Some studies have found no relationship between energy expenditure and reproductive activities, such as visiting rate (Westerterp & Bryant 1984; Bryant 1988; Galbraith *et al.* 1999). However, field metabolic rates (FMR) and nest provisioning rates have been positively correlated in a study by Siegel *et al.* (1999). These relationships, and the one identified in this study, suggest that changes in nest provisioning rates can indeed be a reliable index of reproductive effort (and also reproductive investment) in some species (Daan *et al.* 1996).

Warmed females had a positive energy balance at dawn, relative to controls. The strategy for allocation of this energy probably reflects selection pressures to deliver optimally the energy required by nestlings, with spare time being allocated to other activities which could enhance fitness but which, based on our observations, were not connected to parental duties such as self-feeding (Kacelnik & Cuthill 1990). Energy available to warmed females, surplus to that devoted to provisioning, might have been used to enhance somatic investment. However, this type of response was not detected in this study, where all of the energy 'donated' to the swal-

low was evidently used to increase nest visitation. Indeed, birds not only expended the donated energy, but expended more energy than afforded by the treatment. The disproportionate output response relative to inputs via our treatments implies that the fitness implications of units of energy differed according to whether they represented short-term gains or losses. Alternatively, adult swallows may choose not to, or be obliged not to, balance energy completely in a 24 h period.

If adult swallows maintain an optimal mass (i.e. at dusk or dawn) for brood rearing in order to minimize flight costs over this busy period (Freed 1981; Norberg 1981; Jones 1987; Cavitt & Thompson 1997), then 'donating' energy via treatments could be manifest, via an increased mass, as a greater wing loading at dawn than 'expected'. From this viewpoint, greater reserves at dawn could be regarded as a 'negative' manipulation because flight costs would increase. In practice, there was no difference in mass loss overnight across all three groups, however, so all birds had the same starting mass at dawn, even though there would have been treatment differences in overnight substrate use and water loss. We infer that it was these differences, which proximately gave rise to the observed changes in AEE and associated behaviour, and this did not occur due to an obligatory burden of mass-dependent flight costs.

Although to our knowledge, there are no other studies of the effects of body state manipulations on energy expenditure by breeding birds, analogous experiments on an iguanid, *Uta stansburiana*, allow certain comparisons to be made. Follicle ablation in gravid females, leading to reduced clutch size and mass, was considered to moderate 'foraging effort and yolk synthesis' (Sinervo & DeNardo 1996), which in turn lowered energy expenditure. This allowed the post-laying body condition of females to increase, along with their survival. Equally, ovarian stimulation using exogenous hormones had the reverse effect, with energy expenditure increasing as a result. These responses to manipulations of egg mass comply with ours for swallows, in that experimental reductions and increases in reserves within lizards during breeding had knock-on effects on parental energy expenditure.

This study has shown that the effort devoted to reproduction is state dependent. Even so, it is unclear if physiological state alone provides the cues. Aerial-feeding birds live in a highly variable environment (Martins & Wright 1993) and it has been found that swallows will feed their broods more often in good weather, and also respond to differences in brood size (Bryant & Turner 1982; Jones 1987). Hence, the swallow may adopt a strategy, which allows it to be sensitive to its state, while also responding to other cues, such as chick begging rates and food availability (Godfray 1991). Responses to environmental cues, however, could be relatively unimportant (Cooch & Ricklefs 1994); a pattern consistent with the results of this study. Alternatively, the effects of environmental factors may have been relatively difficult to detect using our study methods. There was also a trend for chilled birds to over-compensate for the manipulation more than warmed ones. This occurred in spite of the smaller temperature difference imposed on chilled birds. This may be due to a ceiling on energy expenditure (Drent & Daan 1980; Hammond & Diamond 1997), which could have placed

an upper limit on the energy expenditure of warmed birds. Therefore, warmed birds may not overcompensate because by doing so they may put their survival at serious risk. By contrast, with a chilled bird, a decrease in expenditure is not liable to the same survival penalty, and so a fall in the energy allocated to breeding should be unconstrained in this respect. If this applies, then symmetrical trade-offs (i.e. H_i) should only be observed at relatively low levels of energy expenditure.

The trade-off identified here implies reproductive effort will be reduced if an animal's survival is jeopardized and vice versa: a life-history response, mediated by an individual's body state. Furthermore, the response of swallows to positive and negative manipulations was large enough to be readily detected, even amongst the variation in energy expenditure related to individual differences and other factors. This, plus the disproportionate expenditure on outputs compared to inputs, suggests that body state not only plays a key role in allocation decisions, but that it is comparable in the scale of its effects to other major influences on energy expenditure of free-living animals (Bryant 1997; Speakman 1997b).

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REFERENCES

- Aschoff, J. & Pohl, M. 1970 Der Ruhezustand von Vögeln als Funktion der Tageszeit der Körpergröße. *J. Ornithol.* **111**, 38–47.
- Bouchon, D. 1991 Biological clock in seasonal reproductive cycle in the ditch shrimp, *Palaemonetes varians* Leach. 2. Ovarian state-dependent responses to non-diel light dark cycles. *J. Exp. Mar. Biol. Ecol.* **146**, 12–26.
- Bryan, S. M. 1996 Energetic constraints on avian incubation: studies of three passerine species. PhD thesis, University of Stirling.
- Bryant, D. M. 1988 Energy expenditure and body mass changes as measures of reproductive costs in birds. *Func. Ecol.* **2**, 23–34.
- Bryant, D. M. 1997 Energy expenditure in wild birds. *Proc. Nutr. Soc.* **56**, 1025–1039.
- Bryant, D. M. & Furness, R. W. 1995 Basal metabolic rates of North-Atlantic seabirds. *Ibis* **137**, 219–226.
- Bryant, D. M. & Turner, A. K. 1982 Central place foraging by swallows: a question of load size. *Anim. Behav.* **30**, 845–856.
- Bryant, D. M. & Westerterp, K. R. 1982 Evidence for individual differences in foraging efficiency among breeding birds: a study of house martins using the doubly labelled water technique. *Ibis* **124**, 187–192.
- Bryant, D. M. & Westerterp, K. R. 1983 Short-term variability in energy turnover by breeding house martins (*Delichon urbica*). *J. Anim. Ecol.* **52**, 905–925.
- Cavitt, J. F. & Thompson, C. F. 1997 Mass loss in breeding house wrens: effects of food supplements. *Ecology* **78**, 2512–2523.
- Cooch, E. G. & Ricklefs, R. E. 1994 Do variable environments significantly influence optimal reproductive effort in birds. *Oikos* **69**, 447–459.
- Daan, S., Deerenberg, C. & Dijkstra, C. 1996 Increased daily work precipitates natural death in the kestrel. *J. Anim. Ecol.* **65**, 539–544.
- Damsgard, B., Arensen, A. M., Baardvik, B. M. & Jobling, M. 1997 State-dependent feed acquisition among two strains of Arctic charr. *J. Fish Biol.* **50**, 859–869.
- Drent, R. H. & Daan, S. 1980 The prudent parent: energetic adjustments in avian breeding. *Ardea* **68**, 225–252.
- Freed, L. A. 1981 Loss of mass in breeding wrens: stress or adaptation? *Ecology* **62**, 1179–1186.
- Galbraith, H., Hatch, J. J., Nisbet, I. T. C. & Kunz, T. H. 1999 Age related changes in efficiency among breeding common terns: measuring energy expenditure using doubly labelled water. *J. Avian Biol.* **30**, 85–96.
- Genstat 5 Committee 1992 *Genstat reference manual, release 3*. Oxford: Clarendon Press.
- Gessaman, J. A. 1987 Energetics. In *Raptor management techniques manual* (ed. B. A. Giron Pendelton, K. W. Cline & D. M. Bird), pp. 289–320. Washington, DC: National Wildlife Federation.
- Godfray, H. C. J. 1991 Clutch size. *A. Rev. Ecol. Syst.* **22**, 409–429.
- Godfrey, J. D. & Bryant, D. M. 2000 State-dependent behaviour and energy expenditure: an experimental study of European robins on winter territories. *J. Anim. Ecol.* **69**, 301–313.
- Hammond, K. A. & Diamond, J. 1997 Maximal sustained energy budgets in humans and animals. *Nature* **386**, 457–462.
- Henderson, I. G. & Hart, P. J. B. 1993 Provisioning parental effort and reproductive success in jackdaws (*Corvus monedula*). *Ornis Scand.* **24**, 142–148.
- Houston, A. I. & McNamara, J. M. 2000 *Models of adaptive behaviour: an approach based on state*. Cambridge University Press.
- Jacobsen, K. O., Erikstad, K. E. & Saether, B. E. 1995 An experimental study of the costs of reproduction in the kittiwake *Rissa tridactyla*. *Ecology* **76**, 1636–1642.
- Jones, G. 1987 Parent-offspring resource allocation in swallows during nestling rearing: an experimental study. *Ardea* **75**, 145–168.
- Kacelnik, A. & Cuthill, I. C. 1990 Central place foraging in starlings (*Sturnus vulgaris*). II. Food allocation to chicks. *J. Anim. Ecol.* **59**, 653–674.
- Kendeigh, S. C., Dolnik, V. R. & Savrilov, V. M. 1977 Avian energetics. In *Granivorous birds in ecosystems*. (ed. J. R. Pinowski & S. C. Kendeigh), pp. 129–204, 345–377. Cambridge University Press.
- Krementz, D. G. & Pendelton, G. W. 1990 Fat scoring: sources of variability. *Condor* **92**, 500–507.
- Kruuk, L. E. B., Clutton-Brock, T. H., Albon, S. D., Pemberton, J. M. & Guinness, F. E. 1999 Population density affects sex ratio variation in red deer. *Nature* **399**, 459–461.
- Lifson, N. & McClintock, R. M. 1966 The theory and use of the turnover rates of body water for the measurement of energy and material balance. *J. Theor. Biol.* **12**, 46–74.
- Macauley, E. D. M., Tatchell, G. M. & Taylor, R. 1988 The Rothamsted insect survey '12 metre' suction trap. *Bull. Entomol. Res.* **78**, 121–129.
- McNamara, J. & Houston, A. I. 1992 State-dependent life history and its implications for optimal clutch size. *Evol. Ecol.* **6**, 170–185.
- McNamara, J. M. & Houston, A. I. 1996 State-dependent life histories. *Nature* **380**, 215–221.
- Martins, T. L. F. & Wright, J. 1993 Cost of reproduction and

- allocation of food between parent and young in the swift, *Apus apus*. *Behav. Ecol.* **4**, 213–223.
- Minitab, Inc. 1997 *Minitab for Windows release 11.2 user guide*. State College Pennsylvania: Minitab Inc.
- Norberg, R. A. 1981 Temporary weight decline in breeding birds may result in more fledged young. *Am. Natural.* **118**, 838–850.
- Nur, N. 1984 The consequences of brood size for breeding blue tits. 1. Adult survival, weight change and the cost of reproduction. *J. Anim. Ecol.* **53**, 479–496.
- Nur, N. 1986 Is clutch size variation in the blue tit (*Parus caeruleus*) adaptive? *J. Anim. Ecol.* **55**, 983–999.
- Olsson, O. 1997 Clutch abandonment: a state-dependent decision in king penguins. *J. Avian Biol.* **28**, 264–267.
- Partridge, L. 1992 Measuring reproductive costs. *Trends Ecol. Evol.* **7**, 99–100.
- Partridge, L. & Harvey, P. H. 1985 Evolutionary biology—costs of reproduction. *Nature* **316**, 20.
- Patterson, H. D. & Thompson, R. 1971 Recovery of interblock information when block sizes are unequal. *Biometrika* **58**, 545–554.
- Scott, I. A., Mitchell, P. I. & Evans, P. R. 1995 The reliability of fat scores as predictors of the mass of fat carried by individual birds. *Ardea* **83**, 359–364.
- Siegel, M. I., Weathers, W. W. & Bessinger, S. R. 1999 Assessing parental effort in a neotropical parrot: a comparison of methods. *Anim. Behav.* **57**, 73–79.
- Sinervo, B. & DeNardo, D. F. 1996 Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* **50**, 1299–1313.
- Speakman, J. 1997a *Doubly labelled water: theory and practice*. London: Chapman & Hall.
- Speakman, J. 1997b Factors influencing the daily energy expenditure of small mammals. *Proc. Nutr. Soc.* **56**, 1119–1136.
- Stearns, S. C. 1992 *The evolution of life histories*. Oxford University Press.
- Tatner, P. & Bryant, D. M. 1989 The doubly labelled water technique for measuring energy expenditure. In *Techniques in comparative respiratory physiology* (ed. C. R. Bridges & P. J. Butler), pp. 77–112. Cambridge University Press.
- Van Noorwijk, A. J., Van Balen, J. H. & Scharloo, W. 1981 Genetic and environmental variation in clutch size of the great tit (*Parus major*). *Neth. J. Zool.* **31**, 342–372.
- Westerterp, K. R. & Bryant, D. M. 1984 Energetics of free-existence in swallows and martins (Hirundinidae) during breeding: a comparative study using doubly labelled water. *Oecologia* **62**, 376–381.
- Williams, G. C. 1966 *Adaptation and natural selection*. Princeton University Press.

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