

## Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*

Olivier Chastel, André Lacroix and Marcel Kersten

Chastel, O., Lacroix, A. and Kersten, M. 2003. Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*. – J. Avian Biol. 34: 298–306.

We measured thyroid hormone (T3) levels and energy expenditure of pre-breeding house sparrows *Passer domesticus* in relation to the timing of breeding and reproductive success. The onset of reproduction was synchronised in two waves, separated by a three-week interval. On an annual basis, early breeders (birds that bred for the first time during the first wave) made significantly more breeding attempts, laid significantly more egg and raised 2.3 times more chicks to fledging than late breeders (birds that bred for the first time during the second wave). By the end of March, about one month before the first egg was laid in the population, plasma titres of testosterone in males and estradiol in females were still low and did not differ between early and late breeders. However, birds that subsequently bred early had higher titres of plasma triiodothyronine (T3) than birds that started to breed late. We show for the first time in a free-living bird population that Basal Metabolic Rate (BMR) is positively correlated with the plasma titre of T3. Differences in plasma T3 accounted for 48% of the inter-individual variation in BMR. Elevated T3 levels indicate that energy requirements increase prior to breeding. Although early breeding appears to be advantageous in terms of the number of offspring raised on an annual basis, the increased energy requirements prior to breeding are thought to delay the onset of reproduction in those birds that cannot afford the additional energy expenditure early in the season.

*O. Chastel (correspondence), A. Lacroix and M. Kersten, CNRS, UPR 1934, Centre d'Etudes Biologiques de Chizé, 79360 Villiers-en-Bois, France. E-mail: chastel@cebc.cnrs.fr. Present address of M. Kersten: Centre for Ecological and Evolutionary studies, Faculty of Mathematics and Natural Sciences, University of Groningen, P.O. box 14, 9750 AA Haren, The Netherlands.*

The timing of laying is one of the reproductive traits with the greatest impact on fitness and a general feature of birds reproducing in a seasonal environment is a steady seasonal decline in reproductive success (Perrins and McCleery 1989, Daan et al. 1990a, Nilsson 1999). To fledge their chicks successfully adult birds have to adjust the timing of laying to food availability (Lack 1968), and in temperate areas, birds match the timing of breeding with a short period in the year when food is most abundant. Several supplementary feeding experiments have generally resulted in an advancement of laying date (reviewed in Nager et al. 1997) suggesting that food availability early in the season might limit egg formation through an energetic constraint (*constraint*

*hypothesis*; Lack 1966, Perrins 1970), or acts as a cue to anticipate trophic conditions when birds will have to rear their young (*anticipation hypothesis*; Lack 1954).

At the proximate level, few studies have measured the energetic cost of getting physiologically prepared for breeding to explain variation in a fitness related trait such as the timing of egg laying (Stevenson and Bryant 2000, Nilsson and Råberg 2001). Most studies addressing the relation between energy expenditure and reproductive decisions have focused on the nestling period (Sanz and Tinbergen 1999, Tinbergen and Verhulst 2000, Nilsson 2002) because the high levels of parental activity associated with food provisioning suggest that daily energy expenditure is maximal during

this phase of the breeding cycle (Drent and Daan 1980). However, the pre-breeding period can also be viewed as an energy demanding period since the preparations for breeding (e.g. nest or mate acquisition, egg or gonad formation) are likely to increase energy requirements as well (Ricklefs 1974, Carey 1996, Nilsson and Råberg 2001). For example, measures of field metabolic rate (FMR) of egg-laying female passerines, which include the cost of foraging, have reported rates that are comparable to FMR during the chick-rearing period (Bryant and Tatner 1988, Ward 1996). Since food availability is still restricted early in the season, and energy expenditure has to be balanced by food intake, slightly elevated rates of energy expenditure prior to breeding might be difficult to sustain at this time of the year. In the great tit *Parus major*, an energy trade-off between reproduction and maintenance that occurs during cold weather has been reported, suggesting a thermal constraint on the timing of egg-laying (Stevenson and Bryant 2000). Such a trade-off may operate through an allocation process between egg production and thermoregulation processes (Stevenson and Bryant 2000).

The possible metabolic and thermoregulatory nature of the physiological constraints acting on the timing of laying suggest that several hormones are probably involved, with likely candidates including the thyroid hormones. Thyroid hormones and especially triiodothyronine (T3) plays an important role in substrate metabolism and thermogenesis and many specific aspects of thyroid hormone-triggered metabolic effects have been established in birds (McNabb 2000). Administration of exogenous thyroid hormones stimulates and thyroidectomy or goitrogen administration depresses oxygen consumption (McNabb 2000). In developing birds, there are positive correlations between periods of high plasma and high metabolic activity (Christensen et al. 1982), and positive correlation between oxygen consumption and T3 levels have been established in young chickens (Bobek et al. 1977). Measuring individual variations in T3 levels early in the season could therefore shed some light on the energetic constraints acting on the timing of reproduction.

In this study we investigated the relation between T3 levels and energy expenditure of pre-breeding house sparrows *Passer domesticus* in relation to the onset of breeding. The aim of this study was to examine whether individual variations in pre-breeding T3 levels and pre-breeding energy expenditure may parallel individual variations in laying date.

We will show that one month before egg-laying the concentration of plasma T3: (1) differ between early and late breeders, and (2) is positively correlated with basal metabolic rate (BMR) in individual birds. Since early breeders raised more offspring on an annual basis than late breeders did, the results suggest that

birds that are unable to sustain elevated rates of energy expenditure early in the season will delay the onset of breeding with a subsequent fitness penalty.

## Methods

### Study population

The study was conducted from March to August 1997 on a free-living population of house sparrows breeding on the compounds of the Centre d'Études Biologiques de Chizé, France (46°09'N, 0°24'W). Virtually all birds in the population were captured with mistnets and wire traps between March and May 1997 and equipped with a numbered aluminum ring and a unique combination of three colour-rings to facilitate individual identification in the field (Chastel and Kersten 2002).

### Reproductive success

Most house sparrows bred in one of the 70 nestboxes installed since 1992 and nestbox owners were identified from their colour-rings using telescopes. From early April onwards, nestbox contents were checked every two days to determine laying date, clutch size, number of hatchlings and number of chicks fledged. Incubation lasts on average 12 days ( $\pm 1$  day) since the last laid egg. Since chicks fledged when they were 12–14 days old, all nestlings were ringed and weighed at an age of 11 days. We used the number of chicks surviving until 11 days after hatching as a measure of reproductive success.

### Blood collection

Plasma titres of the following hormones were determined in pre-breeding house sparrows captured between 20 and 29 March 1997: 3,3',5-triiodo-L-thyronine (T3), testosterone (males only) and 17 $\beta$ -estradiol (females only). During the pre-breeding period, house sparrows were captured with mistnets in the evening at their roosting sites in the Centre d'Études Biologiques de Chizé. During the breeding season, we captured each parent with an electronic trap installed at the entrance of the nestbox when the nestlings were 10–11 days old, that is 1–3 days before fledging. Although this method was highly effective, it was impossible to use at earlier phases of the breeding cycle, because house sparrows turned out to be very sensitive to disturbance and easily abandoned eggs or small chicks when captured at the nest. Thus, in order to describe changes in plasma T3 levels throughout the breeding season, we had to rely on random catches

with mistnets during incubation and early chick rearing (from hatching to day 6). Immediately after capture, a small sample of blood (100–200  $\mu$ l) was collected in a 200  $\mu$ l heparinized micro tube from the brachial vein following puncture with a 27-gauge needle. Blood samples were immediately centrifuged at 2000 rpm for 8 min in order to separate plasma from blood cells. Plasma was stored at  $-20^{\circ}\text{C}$  until final analysis. The only way to minimize a possible effect of diurnal variation in plasma T3 titres (Singh et al. 1967, Newcomer 1974) is to collect blood sample at a standard time. All our blood samples were collected between 16:00 and 20:00 h, which coincides with the expected daily maximum of plasma T3 (Klandorf et al. 1978).

### Radioimmunoassays

Hormone titres were determined by radioimmunoassay using antibodies specific for testosterone and estradiol, respectively. Testosterone and estradiol antisera were kindly provided by Dr. Gérard Picaper (Medecine nucléaire, CHU la Source, Orléans, France) and by Dr. Jean Fiet (Hôpital St. Louis, Paris, France), respectively. Testosterone was assayed for males and estradiol for females. Testosterone and estradiol were assayed in two separate assays and the assay procedure was the same for the two steroids. Each hormone was extracted from a 50  $\mu$ l plasma sample in diethyl-ether and determined in duplicates in one single assay each. Duplicate aliquot of the extracts redissolved in 0.01 M phosphate-buffered saline (pH 7.4) containing 0.1% bovine albumine (PBS-BSA) were incubated overnight at  $4^{\circ}\text{C}$  with ca. 9000 cpm of the appropriate  $^3\text{H}$ -steroid and anti-serum. Intra-assay coefficients of variation were 7% for testosterone and 4% for estradiol ( $n = 6$  duplicates for each hormones). The lowest concentration detectable was 0.078 ng/ml for testosterone and 0.04 ng/ml for estradiol. Total plasma T3 was determined in two assays without extraction in the presence of 8-anilino-1-naphtalen sulfonic acid (ANS) on duplicate samples of 20  $\mu$ l plasma. We obtained  $^{125}\text{I}$ -T3 from CIS Biointernational (OCPE89 T3, 150KBq). Standard T3 (T2877) and T3-binding antisera developed in rabbit (T2777) were obtained from Sigma Chem. Comp. Reagents were diluted in 0.075 M barbital buffer (pH 8.6) which contained 0.1% Azide and 26% normal rabbit serum. Appropriately diluted sheep antirabbit delta-globulin serum was used to precipitate  $^{125}\text{I}$ -T3 bound to antibodies. Pooled plasma of different house sparrows produced a dose-response curve that paralleled the T3 standard curve. The lowest concentration detectable was 0.038 ng/ml. The intra-assay coefficient of variation was 2% ( $n = 4$  duplicates), while the inter-assay coefficient of variation amounted to 16.2% ( $n = 5$  duplicates).

### Oxygen consumption

Basal metabolic rate was determined for adult birds in March by open circuit respirometry. House sparrows were captured in the evening at their roosting sites in the Centre d'Études Biologiques de Chizé. To avoid possible disturbance of metabolic measurements on future reproductive output, the metabolic study was conducted on a sub-sample of birds roosting in a building situated near the study plot where nest boxes have been installed. Birds ( $n = 22$ , 13 males and 9 females) were captured using mistnets and a small blood sample (50  $\mu$ l) was taken for T3 assay. A few minutes later, birds were transported to the lab and placed into a metabolic chamber with an internal volume of 8.0 l. Air temperature within the metabolic chamber was controlled at  $23\text{--}24^{\circ}\text{C}$ , which is at the lower end of the thermoneutral zone of house sparrows (Kendeigh et al. 1977). Atmospheric air was pumped through the metabolic chamber at a rate of 32–38 l/h, measured with a Platon Model 2044 flowmeter and converted to standard values (STPD). Oxygen concentration was measured with a Servomex Series 1100 paramagnetic gas analyser (resolution 0.01%) and carbon dioxide concentration with a Servomex Model 1410 infrared gas analyser (resolution  $\leq 0.02\%$ ). The rates of oxygen consumption and carbon dioxide production were calculated according to the appropriate equations given by Hill (1972). Birds soon became quiet after entering the darkened metabolic chamber and remained so throughout the night. RQ-values gradually decreased to stabilise after 1–3 h at a value close to 0.7, indicating that the bird had reached a post-absorptive state and that fat was the main substrate being metabolised. BMR was calculated from the average rate of oxygen consumption between 2300 and 0600 hrs, using an energy equivalent of 20 kJ/l  $\text{O}_2$ . Birds were released after measurements.

All measurements are expressed as average  $\pm$  one standard error, unless otherwise indicated. Statistical analysis were performed using SYSTAT 7.0 (Wilkinson 1997). All statistical tests are two-tailed, and probability levels  $< 0.05$  were considered significant. Bonferroni corrections were used in case of multiple comparisons.

## Results

### The onset of breeding and reproductive success

Reproduction was highly synchronised. A first wave of clutches was produced by the end of April and birds that laid these clutches were called early breeders (Fig. 1). Towards the end of May, a second wave of clutches started (Fig. 1). In this second wave, 17 out of 26 clutches were produced by birds which had their first attempt of the year and these birds were called late breeders. Daily observations of colour-ringed individu-

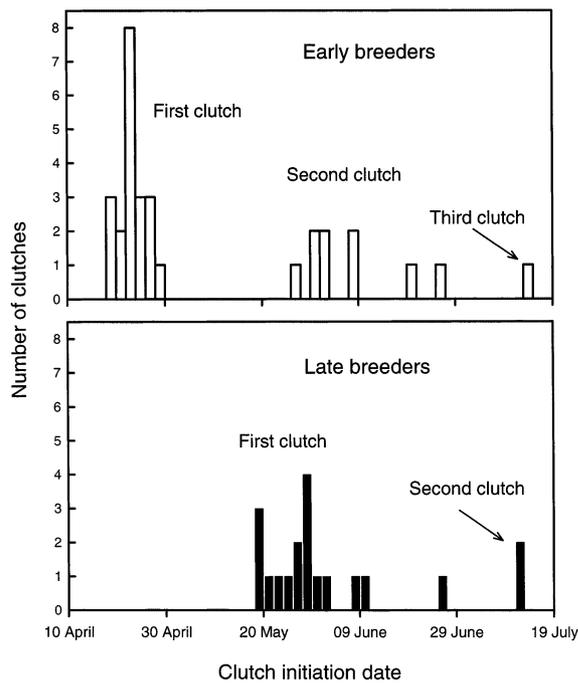


Fig. 1. Clutch initiation dates of house sparrows at Chizé, western France, in 1997. Open bars refer to first and subsequent clutches of early breeders; black bars represent first and second clutches of late breeders.

als confirmed that these late breeding birds occupied and defended their own nestboxes throughout April and May, nests were constructed but eggs were not produced before the end of May. Within this second wave of laying, 9 clutches were second clutches of early breeders and were produced around early June. By mid-July, late breeders produced their second clutches and a third clutch of early breeders was laid.

Considering the first clutch only, clutch size was similar between early and late breeders (early breeders:  $4.72 \pm 0.83$  eggs,  $n = 20$ ; late breeders:  $4.74 \pm 0.93$ ,  $n = 17$ ,  $t$ -test,  $t = 0.016$ ,  $P = 0.98$ ). On an annual basis, early breeders made significantly more breeding attempts, laid significantly more eggs than late breeders (Table 1) and, as a consequence, early breeders produced 2.3 times as many fledglings as late breeders (Table 1).

Table 1. Annual reproductive output of house sparrows, at Chizé, western France in 1997. Early breeders produced their first egg before 29 April, while late breeders produced their first egg after 19 May. Probability values were adjusted for Bonferroni tests.

	Early breeders			Late breeders			t-test	df	P
	Average	S.E.	n	Average	S.E.	n			
Attempts	1.75	0.13	12	1.12	0.12	8	3.29	18	0.012
Eggs laid	8.75	0.84	12	4.87	0.35	8	4.27	18	0.002
Chicks fledged	6.00	0.99	12	2.62	0.50	8	3.04	18	0.024

### Testosterone and estradiol levels during the pre-breeding period

In March, all males sampled, including those that did not enter the breeding population, showed detectable testosterone titres (average:  $0.85 \pm 0.08$  ng/ml,  $n = 67$ ; lowest measurement: 0.19 ng/ml). Among males that subsequently bred, testosterone levels in March did not differ between early breeding males ( $1.02 \pm 0.26$  ng/ml,  $n = 11$ ) and late breeding males ( $1.35 \pm 0.29$  ng/ml,  $n = 8$ ,  $t$ -test,  $t = 0.84$ ,  $P = 0.41$ ). Testosterone was not correlated with plasma T3 ( $r = 0.31$ ,  $n = 19$ ,  $P = 0.20$ ).

In March, all females sampled, including those that did not enter the breeding population, showed detectable estradiol titres (average:  $0.41 \pm 0.02$  ng/ml,  $n = 40$ ; lowest measurement: 0.203 ng/ml). Estradiol levels in March did not differ between early breeding females ( $0.37 \pm 0.03$  ng/ml,  $n = 8$ ) and late breeding females ( $0.35 \pm 0.07$  ng/ml,  $n = 6$ ,  $t = 0.271$ ,  $P = 0.79$ ). Estradiol was not correlated with plasma T3 ( $r = 0.33$ ,  $n = 14$ ,  $P = 0.25$ ).

### Body mass

Body mass in March was significantly higher in males than in females (Sex:  $F_{1,34} = 8.78$ ,  $P = 0.01$ ), but did not differ between early and late breeders (Early/Late:  $F_{1,34} = 1.53$ ,  $P = 0.22$ ; interaction Sex  $\times$  Early/Late:  $F_{1,34} = 3.00$ ,  $P = 0.09$ ; Fig. 2). Body mass was not significantly correlated with the level of plasma T3 (sexes combined:  $r = 0.20$ ,  $n = 38$ ,  $P = 0.22$ ). Male body mass was not significantly correlated with testosterone level ( $r = 0.35$ ,  $n = 19$ ,  $P = 0.14$ ). Similarly, female body mass was not significantly correlated with estradiol level ( $r = 0.22$ ,  $n = 14$ ,  $P = 0.46$ ).

### Triiodothyronine (T3)

All birds sampled showed detectable T3 titres. Plasma T3 of house sparrows captured by the end of March (including those that did not enter the breeding population) averaged  $0.48 \pm 0.03$  ng/ml ( $n = 57$ ). Plasma T3 did not increase or decrease over the 10-day measurement period (slope:  $-0.004$  (ng/ml/day),  $r^2 = 0.01$ ,  $n = 57$ ,  $P > 0.1$ ).

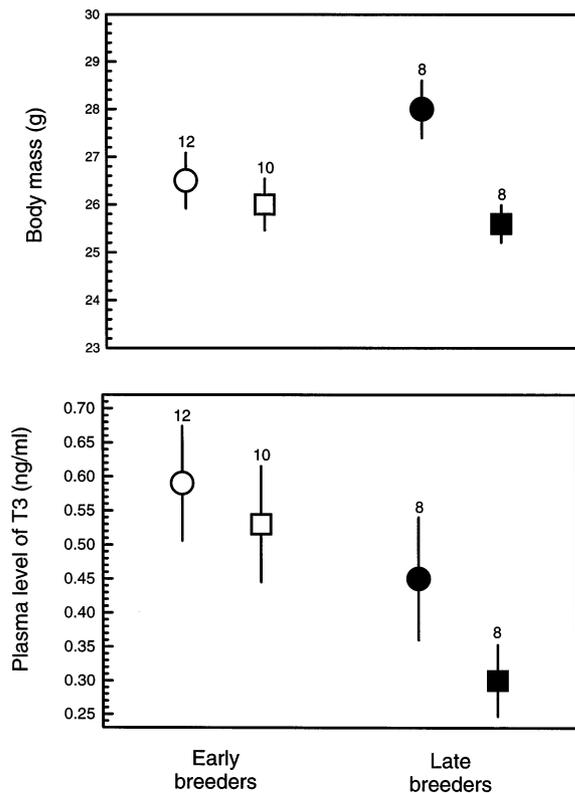


Fig. 2. Body mass and plasma levels of T3 of early (open symbols) and late breeding (black symbols) house sparrows in March 1997. Circles refer to males, squares to females. Sample sizes are indicated at the top of error bars which represent one standard error.

Although plasma T3 in males was slightly higher than in females, this trend was not statistically significant (Sex:  $F_{1,34} = 1.37$ ,  $P = 0.25$ ; Fig. 2). Plasma T3 in March was significantly higher in early breeders than in late breeders (Early/Late:  $F_{1,34} = 4.7$ ,  $P = 0.04$ ; interaction Sex  $\times$  Early/Late:  $F_{1,34} = 0.35$ ,  $P = 0.56$ ; Fig. 2). In early breeders, plasma T3 increased throughout April to an average value of  $1.14 \pm 0.10$  ng/ml ( $n = 8$ ) during incubation (Fig. 3). Late breeders attained a similar level by the end of May (Fig. 3).

#### Relation between plasma T3 and BMR

BMR in March averaged  $0.23 \pm 0.01$  W ( $n = 22$ ). BMR of adult house sparrows in March increased linearly with the level of plasma T3 (regression coefficient:  $r = 0.62$ , variance explained: 38%,  $n = 22$ ,  $P < 0.01$ ; Fig. 4). On the other hand, body mass alone had no significant effect on BMR (regression coefficient:  $r = 0.36$ ,  $n = 22$ ,  $P = 0.103$ ). A multiple linear regression with plasma T3 and body mass (BM) as independent variables explained 48% (T3, partial regression coefficient:  $r = 0.58$ , variance explained: 34%,  $P = 0.004$ ; BM, par-

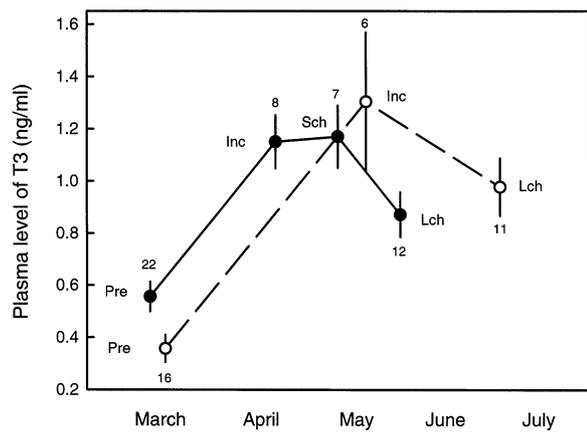


Fig. 3. Plasma T3 levels of early (open symbols) or late (black symbols) breeding adult house sparrows at different stages of the breeding cycle. Shown are average values representing: Pre-breeding birds (Pre), birds incubating a clutch (Inc), birds tending 1–6 day old chicks (Sch) and birds tending 10–12 days old chicks (Lch). There were no data for late breeding birds tending 1–6 day old chicks. Error bars represent one standard error, numbers at the top denote sample sizes.

tial regression coefficient:  $r = 0.14$ ,  $P = 0.04$ ) of the variance in measured BMR:

$$\text{BMR (W)} = -0.146 + 0.012 \times \text{body mass (g)} + 0.145 \times \text{T3 (ng/ml)} \quad (1)$$

On average, females appeared to have a slightly lower BMR than males ( $t = 2.06$ ,  $P = 0.053$ ), but this was mainly due to a difference in body mass. Sex did not contribute significantly to the explained variance (ANOVA, Sex:  $F_{1,19} = 2.08$ ,  $P = 0.17$ ).

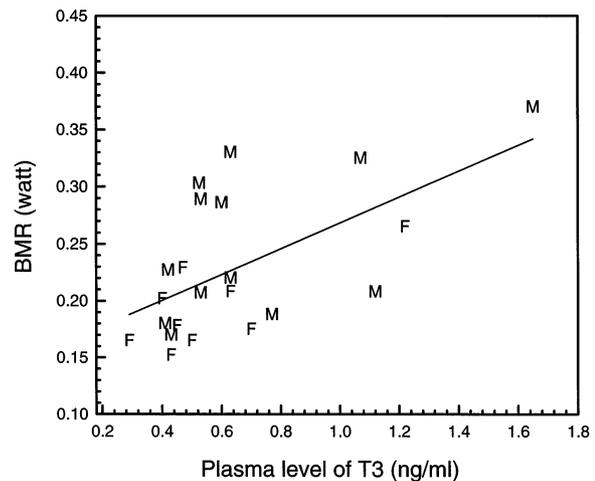


Fig. 4. Relation between BMR and plasma T3 in house sparrows prior to breeding. M denotes males and F denotes females.

## Discussion

### T3 and the rate of energy expenditure

The role of thyroid hormones in the regulation of energy metabolism in birds is well established (Hazelwood 1972, Jansky 1973). Thyroxine (T4) secreted by the thyroid gland is peripherally converted into triiodothyronine (T3), which is now regarded as the metabolically active form of thyroid hormones (Bobek et al. 1980, May 1982). Although T4 may well be involved in the regulation of other physiological processes, there is no evidence that T4 by itself affects the rate of energy expenditure. A thermogenic effect of T3 has been demonstrated in domesticated bird species (Bobek et al. 1977, 1980, Hwen-Bo et al. 1990). The relation is causal since metabolic rate increases shortly after experimental administration of T3, while thiouracil (which blocks T3 action) causes metabolic rate to decrease (McNabb 2000). The turnover rate of T3 in birds is relatively high because birds, in contrast to mammals, lack a specific T3-transporting protein in their blood, which makes T3 a potentially much more powerful agent in birds than in mammals (McNabb 2000). Evidence that T3 is involved in the regulation of energy expenditure in non-domesticated bird species, either captive or free-living, is circumstantial (Rising and Hudson 1974). In this study we show for the first time in a free-living bird population that part of the inter-individual variation in BMR is accounted for by differences in plasma T3.

Daily energy expenditure of free-living birds and sustainable rates of energy expenditure vary in parallel with BMR, both inter-specifically (Kersten and Piersma 1987, Daan et al. 1990b, Lindström and Kvist 1995) and intra-specifically (Cooper and Swanson 1994, Dutenhoffer and Swanson 1996, Liknes and Swanson 1996). Consequently, differences in plasma T3 level are likely to reflect different levels of daily energy expenditure between individual birds. Traditionally, T3 has been associated with cold acclimatization (Burke et al. 1977, Dawson et al. 1992) and hyperphagia (Schildmacher and Rautenberg 1952, Chandola and Pathak 1980, Pathak and Chandola 1982, Stokkan et al. 1985). Some studies report high values of T3 during the breeding season, ruffed grouse *Bonassa umbellus* (Garbutt et al. 1979), white-crowned sparrow *Zonotrichia leucophrys* (Smith 1982) and spotted munia *Lonchura punctulata* (Pathak and Chandola 1983), but none of these authors associated T3 explicitly with reproduction. We presume that the effect of T3 on laying date in the house sparrow is indirect, acting via its effect on BMR. We propose that elevated T3-levels are associated with any period of increased energy expenditure in the annual cycle, be it cold stress or accumulation of energy reserves or reproduction.

### T3 and the onset of breeding

Plasma T3 levels of early and late breeders differed already by the end of March, i.e. one month before early breeders produced their first egg. This early in the season, plasma levels of testosterone and estradiol were still low. Both these reproductive hormones show a marked peak just prior to egg-laying (Hegner and Wingfield 1990). This suggests that metabolic preparations for breeding precede sexual activity. In March, according to Eq. (1), the higher level of plasma T3 of early breeders represents only a 12% higher estimated BMR compared to that of late breeders. However, T3 levels are on the rise in March, attaining 1 ng/ml around egg-laying. Early breeders reach this level in April, late breeders in May. According to Equation (1), this level of plasma T3 corresponds to an estimated BMR of 0.32 W, which is 42% higher than the BMR of late breeders in March. Assuming that daily energy expenditure increases proportionally to BMR, the energy requirements of early breeders would be 10–40% higher than that of late breeders. We interpret the observed inter-individual differences of plasma T3 as a reflection of the more or less advanced state of the metabolic preparations for breeding of the individuals involved.

The relationships between plasma T3, BMR and laying date demonstrated at the individual level, also hold at the scale of the entire population. BMR in March averaged  $0.23 \pm 0.01$  W. This is considerably lower than predicted or measured values, which range from 0.34 to 0.46 W (Aschoff and Pohl 1970, Kendeigh et al. 1977). This low BMR coincides with low levels of plasma T3, which averaged  $0.50 \pm 0.27$  ng/ml in our population. Average values of both plasma T3 and BMR in the study population were low compared to other house sparrow populations, for example, in a population of house sparrows in Oregon, plasma T3 was in the order of 3–4 ng/ml during winter (Smith 1982). Comparisons of hormone titres between studies are hampered by the fact that radioimmunoassays often differ with respect to the proportion of the absolute amount of hormones they measure. This is especially true for heterologous assays of protein hormones, but inter-assay variability is much smaller among non-heterologous assays of steroid hormones and thyroid hormones, which have an identical molecular structure throughout the vertebrate phylum. Given the sheer magnitude of the difference in plasma T3 titres between our study and that of Smith (1982), we think it is likely that the uncharacteristically low BMR in the study population is associated with low plasma T3 titres. The onset of breeding in the study population is delayed as well (Fig. 5). The average laying date of the first egg in the population is 18 April, which is 18 days later than predicted at this latitude. In fact, this corresponds to the typical laying date for southern Sweden, which is

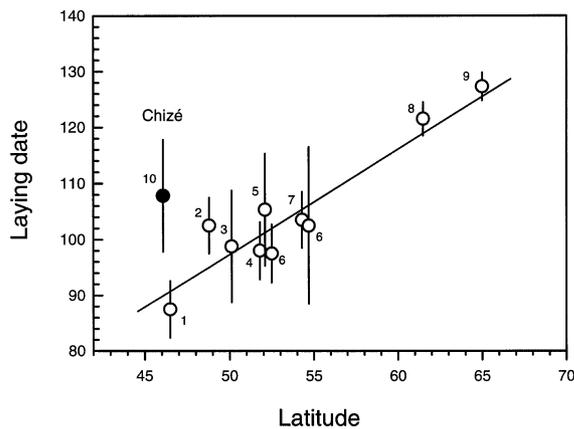


Fig. 5. Laying date (Julian) of house sparrows in lowland Europe (altitude under 700 m) in relation to latitude (open symbols). Laying date of the first egg in the population increased linearly with latitude according to the relation:  $Y = 1.31 + 1.924X$ ;  $r^2 = 0.89$  (Student *t*-test:  $t = 8.04$ ,  $n = 10$ ,  $P < 0.001$ ). Vertical bars denote one standard deviation. In the study population at Chizé (black symbol) the first egg is produced around 18 April (95% conf. int.: 7–30 April, data from 1997 to 1999), which is significantly later than the predicted laying date of 30 March at this latitude ( $P < 0.05$ ). Numbers indicate the following data sources: (1) Ion 1973, Moldova; (2) Barloy 1966, France; (3) Mackowicz et al. 1970, Poland; (4) Seel 1968, England; (5) Pinowska and Pinowski 1977, Poland; (6) Wieloch and Fryska 1975, Poland; (7) Deckert 1969, Germany; (8) Rassi, in Kendeigh et al. 1977, Finland; (9) Alatalo 1975, Finland; (10) this study, France.

about 1100 km further north. We can only speculate about the cause of this considerable retardation, but the important point is that the delayed laying date coincides with both a low level of plasma T3 and a lower than expected BMR.

### Pre-breeding energy, laying date and annual reproductive success

On an annual basis, early breeders raised 2.3 times as many fledglings as late breeders. The number of breeding attempts per year could have been biased by our research activities. House sparrows turned out to be very sensitive to disturbances and some parents abandoned their brood after they were captured in the nestbox when the chicks were 11 days old. We suspect that our activities may have prevented a number of birds from re-nesting in the same nestbox. Some birds switched to an adjacent nestbox, but we cannot exclude that a few others produced a subsequent clutch under the roof. Although the number of breeding attempts may be underestimated, it is unlikely that this would affect the conclusion that early breeders produce more fledglings per season than do late breeders.

This suggests that natural selection should favour early breeding. However, early reproduction does not seem to be affordable by all individuals. In this paper

we show that the onset of reproduction is associated with an elevated rate of energy expenditure, reflected by high levels of plasma T3, both at the individual and at the population level. Increased energy expenditure is affordable only if this can be balanced by food intake. Because individual birds probably differ in their ability to extract food from the environment, some birds are able to cope with these costs, while others are not. Food availability increases as the season advances, enabling less skilful foragers to cope with the elevated rate of energy expenditure at a later date. This suggests that inter-individual differences in foraging skills are responsible for differences in the onset of reproduction, enabling the more skilful birds to increase their plasma T3 titre and hence their rate of energy expenditure at an earlier date. This interpretation is consistent with the results of surplus feeding experiments. Increased food availability invariably results in an advancement of laying date, indicating that the timing of reproduction is causally related to food availability (Martin 1987).

In many species, older birds start egg-laying earlier than young birds (reviewed in Perdeck and Cavé 1992). Older or dominant individuals probably have better access to food resources in flocks (Barnard and Sibly 1981, Møller 1987) and therefore may be able to cope with these pre-breeding metabolic costs. For instance, a positive correlation between social dominance and the BMR has been documented in four passerine species (Røskoft et al. 1986, Hogstad 1987, Bryant and Newton 1994).

Individual laying date is determined by the balance between elevated energy expenditure prior to breeding and increasing food availability as the season progresses. So far, reproductive decisions have been investigated by manipulation of food availability (Daan et al. 1989). The causal relation between plasma T3 and BMR opens the possibility to influence reproductive decisions by manipulation of the energy expenditure side of the equation. T3 can be administered orally to elevate BMR but is also often toxic and injection of T3 have very short-lived effects. Possibly, the hormone to use would be T4 because it would provide sustained thyroid hormone exposure with the bird doing its own deiodination to T3 on a sustained basis (Derting 1989). Phenotypic engineering (Ketterson and Nolan 1992, Ketterson et al. 1996), by addition of thyroid hormone or thiouracil (a suppressor of T3, McNabb 2000) to supplemented food, may enable us to modify the energy requirements and investigate the fitness consequences of these modifications.

*Acknowledgements* – We are grateful to Céline Clément, Nicolas Gaidet and Didier Capdeville for continuous help in the field, while Joël Bried and Hervé Lormée assisted at crucial moments. We also express our gratitude to Dr. Gérard Picaper (Medecine nucléaire, CHU la Source, Orléans, France) and Dr. Jean Fiet (Hôpital St. Louis, Paris, France) who kindly provided us with the testosterone and estradiol antisera. Colette Trouvé and Zofia Marillet assisted with the hormone

assays. Guy Merlet was instrumental in adapting the respirometry configuration to measurements of small animals and kept the equipment running. The research benefited greatly from discussions with Rudi Drent, Joost Tinbergen, Christophe Barbraud and Hervé Fritz. We thank two anonymous referees and the editor for helpful comments on the manuscript. M. K. was financially supported by a grant from the Région Poitou-Charentes.

## References

- Alatalo, R. 1975. Varpusen *Passer domesticus* pesimäbiologiasta Oulun Sanginsuussa. – *Lintumies* 10: 1–7.
- Aschoff, J. and Pohl, H. 1970. Der Ruheumsatz von Vögeln als Funktion der Tageszeit und der Körpergrösse. – *J. Orn.* 111: 38–47.
- Barloy, J.-J. 1966. Recherches sur le moineau domestique *Passer d. domesticus* (Linné) 1758: cycle biologique, ecologie, dynamique de population; sa place dans l'avifaune Parisienne. – Ph.D. thesis, Université de Paris, Paris.
- Barnard, C. J. and Sibly, R. M. 1981. Producers and scroungers: a general model and its implication to captive flocks of house sparrows. – *Anim. Behav.* 29: 543–550.
- Bobek, S., Jastrzebski, M. and Pietras, M. 1977. Age related changes in oxygen consumption and plasma thyroid hormone concentration in the young chicken. – *Gen. Comp. Endocrinol.* 31: 169–174.
- Bobek, S., Neizgoda, J., Pietras, M., Kacinska, M. and Ewy, Z. 1980. The effect of acute cold and warm ambient temperatures on the thyroid hormone concentration in blood plasma, blood supply and oxygen consumption in Japanese quail. – *Gen. Comp. Endocrinol.* 40: 201–210.
- Bryant, D.M. and Tatner, P. 1988. Energetics of the annual cycle in dippers, *Cinclus cinclus*. – *Ibis* 130: 17–38.
- Bryant, D. M. and Newton, A. V. 1994. Metabolic costs of dominance in dippers, *Cinclus cinclus*. – *Anim. Behav.* 48: 447–455.
- Burke, N.S., Lisano, M. E. and Kennamer, J. E. 1977. Variations in plasma thyroxine (T4) and triiodothyronine (T3) in eastern wild turkeys. – *J. Wildl. Manage.* 41: 650–656.
- Carey, C. 1996. Female reproductive energetics. – In: Carey, C. (ed.). *Avian energetics and nutritional ecology*. Chapman and Hall, New York, pp. 324–374.
- Chandola, A. and Pathak, V. K. 1980. Premigratory increase in circulating triiodothyronine/thyroxine ratio in the red-headed bunting, *Emberiza bruniceps*. – *Gen. Comp. Endocrinol.* 41: 270–273.
- Chastel, O. and Kersten, M. 2002. Brood size and body condition in the house sparrow *Passer domesticus*: the influence of brooding behaviour. – *Ibis* 144: 284–292.
- Christensen, V. L., Biellier, H. V. and Forward, J. F. 1982. Physiology of turkey embryo during pipping and hatching. III. Thyroid function. – *Poult. Sci.* 61: 367–374.
- Cooper, S. J. and Swanson, D. L. 1994. Seasonal acclimatization of thermoregulation in the black-capped chickadee. – *Condor* 96: 638–648.
- Daan, S., Dijkstra, C., Drent, R. H and Meijer, T. 1989. Food supply and the annual timing of avian reproduction. *Acta XIX Congr. Int. Ornithol.* – Ottawa Press, Ottawa, pp. 392–407.
- Daan, S., Dijkstra, C. and Tinbergen, J. M. 1990a. Family planning in the kestrel *Falco tinnunculus*: the ultimate control of covariation of laying date and clutch size. – *Behaviour* 114: 83–116.
- Daan, S., Masman, D. and Groenewold, A. 1990b. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. – *Am. J. Physiol.* 259: 333–340.
- Dawson, W. R., Carey, C. and Van't Hof, T. J. 1992. Metabolic aspects of shivering thermogenesis in passerines during winter. – *Ornis Scand.* 23: 381–387.
- Deckert, G. 1969. Zur Ethologie und Ökologie des Haussperlings (*Passer d. domesticus* (L.)). – *Beiträge zur Vogelkunde* 15: 1–84.
- Derting, T. L. 1989. Metabolism and food availability as regulators of production in juvenile cotton rats. – *Ecology* 70: 587–595.
- Drent, R. H. and Daan, S. 1980. The prudent parent: energetic adjustments in avian breeding. – *Ardea* 68: 225–252.
- Dutenhoffer, M. S. and Swanson, D. L. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. – *Physiol. Zool.* 69: 1232–1254.
- Garbutt, A., Leatherland, J. F. and Middleton, A. L. A. 1979. Seasonal changes in serum thyroid hormone levels in the ruffed grouse maintained under natural conditions of temperature and photoperiod. – *Can. J. Zool.* 57: 2022–2027.
- Hazelwood, R. L. 1972. The intermediary metabolism of birds. – In: Farner, D. S. and King, J. R. (eds). *Avian biology*, Vol. 2. Academic Press, New York, pp. 472–576.
- Hegner, R. E. and Wingfield, J. C. 1990. Annual cycle of gonad size, reproductive hormones, and breeding activity of free-living house sparrows (*Passer domesticus* (L.)) in rural New York. – In: Pinowski, J. and Summers-Smith, J. D. (eds). *Granivorous birds in the agricultural landscape*. Intecol, Warszawa, pp. 123–135.
- Hill, R. W. 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. – *J. Appl. Physiol.* 33: 261–263.
- Hogstad, O. 1987. It is expensive to be dominant. – *Auk* 104: 333–336.
- Hwen-Bo, J., Muramatsu, T. and Okumura, J. 1990. Relative biopotency of triiodothyronin and of thyroxin for inducing oxygen consumption in young chicks. – *Poultry Sci.* 69: 1027–1028.
- Ion, I. 1973. Biostatistic and ecological researches on the populations of two sparrow species (*Passer d. domesticus* (L.) and *Passer m. montanus* (L.)) in Moldavia. – Ph.D. thesis, Iasi University, Iasi.
- Jansky, L. 1973. Non-shivering thermogenesis and its thermoregulatory significance. – *Biol. Rev.* 48: 85–132.
- Kendeigh, S. C., Dol'nik, V. R. and Gavrilov, V. M. 1977. Avian energetics. – In: Pinowski, J. and Kendeigh, S. C. (eds). *Granivorous birds in ecosystems*. Cambridge University Press, Cambridge, pp. 127–157.
- Kersten, M. and Piersma, T. 1987. High levels of energy expenditure in shorebirds: metabolic adaptations to an energetically expensive way of life. – *Ardea* 75: 175–187.
- Ketterson, E. D. and Nolan Jr., V. 1992. Hormones and life histories: an integrative approach. – *Am. Nat.* 140S: 33–62.
- Ketterson, E. D., Nolan Jr., V., Cawthorn, M. J., Parker, P. G. and Ziegenfus, C. 1996. Phenotypic engineering: using hormones to explore mechanistic and functional bases of phenotypic variation in nature. – *Ibis* 138: 70–86.
- Klandorf, H., Sharp, P. J. and Duncan, I. J. H. 1978. Variations in levels of plasma thyroxine and triiodothyronine in juvenile female chickens during 24- and 16-hr lighting cycles. – *Gen. Comp. Endocrinol.* 36: 238–243.
- Lack, D. 1954. *The natural regulation of animal numbers*. – Oxford University Press, Oxford.
- Lack, D. 1966. *Population studies of birds*. – Oxford University Press, Oxford.
- Lack, D. 1968. *Ecological adaptations for breeding in birds*. – Methuen, London.
- Liknes, E. T. and Swanson, D. L. 1996. Seasonal variation in cold tolerance, basal metabolic rate, and maximal capacity for thermogenesis in white-breasted nuthatches *Sitta carolinensis* and downy woodpeckers *Picoides pubescens*, two unrelated arboreal temperate residents. – *J. Avian Biol.* 27: 279–288.
- Lindström, Å. and Kvist, A. 1995. Maximum energy intake rate is proportional to basal metabolic rate in passerine birds. – *Proc. R. Soc. Lond. B* 261: 337–343.

- Mackowicz, R., Pinowski, J. and Wieloch, M. 1970. Biomass production by house sparrow (*Passer d. domesticus* (L.)) and tree sparrow (*Passer m. montanus* (L.)) populations in Poland. – *Ecologia Polska* 18: 465–501.
- Martin, T. E. 1987. Food as a limit on breeding birds: a life-history perspective. – *Ann. Rev. Ecol. Syst.* 18: 453–457.
- May, J. D. 1982. Effect of dietary thyroid hormone on survival time during heat stress. – *Poultry Sci.* 61: 706–709.
- McNabb, F. M. A. 2000. Thyroids. – In: Whittow, G. C. (ed.). *Avian physiology*. Academic Press, San Diego, pp. 461–472.
- Møller, A. P. 1987. Variations in badge size in male house sparrows *Passer domesticus*: evidence for status signalling. – *Anim. Behav.* 35: 1637–1644.
- Nager, R. G., Rüegger, C. and van Noordwijk, A. J. 1997. Nutrient or energy limitation on egg formation a feeding experiment in great tits. – *J. Anim. Ecol.* 66: 495–507.
- Newcomer, W. S. 1974. Diurnal rhythms of thyroid function in chicks. – *Gen. Comp. Endocrinol.* 24: 65–73.
- Nilsson, J. Å. 1999. Fitness consequences of timing of reproduction. – *Proc. 22 Int. Ornithol. Congr.*, pp. 234–247.
- Nilsson, J. Å. 2002. Metabolic consequences of hard work. – *Proc. R. Soc. Lond. B* 249: 259–263.
- Nilsson, J. Å. and Råberg 2001. The resting metabolic cost of egg laying and nestling feeding in great tits. – *Oecologia* 128: 187–92.
- Pathak, V. K. and Chandola, A. 1982. Involvement of thyroid gland in the development of migratory disposition in the redheaded bunting *Emberiza bruniceps*. – *Horm. Behav.* 16: 46–58.
- Pathak, V. K. and Chandola, A. 1983. Seasonal variations in circulating thyroxine and triiodothyronine concentrations in spotted munia *Lonchura punctulata*. – *Gen. Comp. Endocrinol.* 50: 201–204.
- Perdeck, A. C. and Cavé, A. J. 1992. Laying date in the coot: effect of age mate choice. – *J. Anim. Ecol.* 61: 13–20.
- Perrins, C. M. 1970. The timing of birds' breeding seasons. – *Ibis* 112: 242–255.
- Perrins, C. M. and McCleery, R. H. 1989. Laying date and clutch size in the great tit. – *Wilson Bull.* 101: 236–253.
- Pinowska, B. and Pinowski, J. 1977. Fecundity, mortality, numbers and biomass dynamics of a population of the house sparrow *Passer domesticus*. – *Stud. Sparrows* 10: 26–41.
- Ricklefs, R. E. 1974. Energetics of reproduction in birds. – In: Paynter, R. A. (ed.). *Avian energetics*. Nuttall Ornithological Club, Cambridge, MA, pp. 152–292.
- Rising, J. D. and Hudson, J. W. 1974. Seasonal variation in the metabolism and thyroid activity of the black-capped chickadee (*Parus atricapillus*). – *Condor* 76: 198–203.
- Røskaft, E., Jarvi, T., Bakken, M., Bech, C. and Reinertsen, R. E. 1986. The relationship between social status and resting metabolic rate in the great tit *Parus major* and pied flycatcher *Ficedula hypoleuca*. – *Anim. Behav.* 34: 838–842.
- Sanz, J. J. and Tinbergen, J. M. 1999. Energy expenditure, nestling age, and brood size: and experimental study of parental behavior in the great tit *Parus major*. – *Behav. Ecol.* 10: 598–606.
- Schildmacher, H. and Rautenberg, W. 1952. Untersuchungen zur hormonalen Regulierung des Fettwerdens der Zugvögel In Frühjahr. – *Biol. Zentralbl.* 71: 397–405.
- Seel, D. C. 1968. Breeding seasons of the house sparrow and the tree sparrow *Passer* spp. at Oxford. – *Ibis* 110: 129–144.
- Singh, A., Reineke, E. P. and Ringer, R. K. 1967. Thyroxine and triiodothyronine turnover in the chicken and bobwhite and Japanese quail. – *Gen. Comp. Endocrinol.* 9: 353–361.
- Smith, J. P. 1982. Changes in blood levels of thyroid hormones in two species of passerine birds. – *Condor* 84: 160–170.
- Stevenson, I. R. and Bryant, D. M. 2000. Climate change and constraints on breeding. – *Nature* 406: 366–367.
- Stokkan, K. A., Harvey, S., Klandorf, H., Unander, S. and Blix, A.S. 1985. Endocrine changes associated with fat deposition and metabolization in Svalbard ptarmigan (*Lagopus mutus hyperboreus*). – *Gen. Comp. Endocrinol.* 58: 76–80.
- Tinbergen, J. M. and Verhulst, S. 2000. A fixed energetic ceiling to parental effort in the great tit? – *J. Anim. Ecol.* 69: 323–334.
- Ward, S. 1996. Energy expenditure of female barn swallows *Hirundo rustica* during egg formation. – *Physiol. Zool.* 69: 930–951.
- Wieloch, M. and Fryska, A. 1975. Biomass production and energy requirements in populations of the house sparrow (*Passer d. domesticus* (L.)) and tree sparrow (*Passer m. montanus* (L.)) during the breeding season. – *Pol. Ecol. Stud.* 1: 243–252.
- Wilkinson, L. 1997. *Systat 7.0 new statistics*. SPSS Inc., Chicago.

(Received 8 July 1999, revised 29 November 2002, accepted 1 January 2003.)