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**Costs of avian incubation**

de Heij, Maaike Elisabeth

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# No indication for clutch size related daily energy expenditure during incubation in female great tits: do parents compensate?

Maaike E. de Heij, Richard Ubels, G. Henk Visser &  Joost M. Tinbergen

## ABSTRACT

Recent studies show that selection on clutch size also acts during the incubation phase, which may be the result of changes in energy expenditure of the attending parents. To determine whether incubating birds increase their energy expenditure with clutch enlargement, we manipulated clutch size (either enlarged or control) and measured daily energy expenditure (24 h) of free-living female great tits (*Parus major* L.) with doubly labelled water. These measures of energy expenditure were combined with measures on nest attendance of the females. Females incubating control clutches containing on average $9.1 \pm 1.2$ eggs spent $78.4 \pm 9.0$ kJ per 24 h, at an average ambient temperature of $13.3 \pm 1.6$ °C. Daily energy expenditure was strongly related to mean ambient temperature over the 24-h measuring period. Daily energy expenditure of females incubating enlarged clutches was not significantly higher than that of females incubating control clutches. Although power was lacking to reveal any small difference in daily energy expenditure between females of the two treatment categories, we argue that females were most likely compensating for the additional energetic costs of incubating enlarged clutches. Yet, the mechanism by which these female compensate remains unclear, and needs to be the focus of future studies.

## INTRODUCTION

Life-history theory predicts that parents produce the number of offspring that maximises their fitness (Stearns 1992; Roff 1992). In birds, natural selection on parental decisions regarding clutch size may act during egg laying, incubation or nestling phase. In altricial birds, selection on clutch size has long thought to be taking place primarily during the nestling phase (Lindén & Møller 1989; Dijkstra *et al.* 1990; Vanderwerf 1992). Recent studies, however, show reduced local survival for female great tits *Parus major* (Visser & Lessells 2001; chapter 2) in the subsequent breeding season when incubating enlarged clutches. Therefore, the incubation phase cannot be ignored in studies on clutch size decisions.

The mechanism through which clutch enlargement reduces local survival in the subsequent season could be an increase in energetic demand for incubating birds. Generally, two methods are applied to measure energy expenditure: (1) respirometry and (2) doubly labelled water (DLW) technique. Respirometry requires periods of relative inactivity, such as during contact incubation, and is often used under laboratory conditions (but see Haftorn and Reinersten 1985; chapter 6). The DLW – technique, on the other hand, is widely applicable in free-living individuals. All of five studies that use respirometry show that the energetic costs of contact incubation is clutch size related (Biebach 1981; Biebach 1984; Haftorn & Reinertsen 1985; Weathers 1985; chapter 6). Studies that use the DLW technique to determine clutch size related energetic costs during a 24-h period reveal no general patterns (Moreno & Carlson 1989; Moreno *et al.* 1991; Moreno & Sanz 1994; Engstrand *et al.* 2002). Two of the four studies found no effect of clutch size on daily energy expenditure during incubation (Moreno & Carlson 1989; Engstrand *et al.* 2002; for details about the studies see Table 7.1), whereas the other two studies did find an effect (Moreno *et al.* 1991; Moreno & Sanz 1994). The study by Moreno *et al.* (1991) hints that females incubating experimentally enlarged clutches have higher $DEE_{inc}$, whereas the study by Moreno and Sanz (1994) does not find an increase in $DEE_{inc}$ with clutch enlargement but shows a decrease in $DEE_{inc}$ with clutch reduction.

Thus, how the energetic costs of additional eggs during nocturnal incubation relate to energy expenditure over 24-h is not obvious. In response to clutch enlargement birds may increase their daily energy expenditure. Alternatively, birds may compensate for the additional energy demands of enlarged clutches by changing their behaviour during the day with the consequence that the daily energy expenditure remains relatively constant. Birds with experimentally enlarged clutches may change their nest attentiveness (Wiebe & Martin 2000). Larger clutches cool and warm more slowly (Reid *et al.* 2000b), possibly allowing birds to leave the clutch unattended for a longer period. A change in nest attentiveness may also lead to a reduction of the number of recesses, and thereby a reduction in the number of times birds need to rewarm the clutch; rewarming of the clutch is found to

**Table 7.1.** Details of studies on clutch size manipulation and DEE$_{inc}$; 1, species; 2, sample size per manipulation category (R = reduced, C = control, E = enlarged); 3, original clutch size; 4, number of eggs added or removed; 5, effect of clutch enlargement on DEE$_{inc}$; 6, change in behaviour; 7, change in body mass; 8 references.

| 1 | 2 | | | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| | R | C | E | | | | | | |
| white-throated dipper *Cinclus cinclus* | 6 | 17 | 8 | 5 | 1 | no | no | no | Engstrand *et al.* 2002 |
| collared flycatcher *Ficedula albicollis* | 4 | 3 | 5 | 6 | 2 | yes | no | no | Moreno *et al.* 1991 |
| pied flycatcher *Ficedula hypoleuca* | 2 | 2 | 2 | 5-7 | 2 | no | yes[a] | yes[a] | Moreno & Carlson 1989 |
| pied flycatcher *Ficedula hypoleuca* | 7 | 3 | 8 | 5-6 | 1 | yes | - | no | Moreno & Sanz 1994 |
| great tit *Parus major* | - | 14 | 15 | 5-12 | 3 | no | no | no | This study |

[a] measured for a larger sample size

be energetically more costly than maintaining egg temperature (Vleck 1981; Biebach 1986).

In this study, we measured the daily energy expenditure of female great tits *Parus major* with use of the DLW technique (DLW), incubating either experimentally enlarged clutches or control clutches. Additionally, we measured nest attentiveness of these females to determine whether birds change behaviourally in reaction to the experimental treatment. The great tit was used as model species, because in this species only one of the parents – the female – incubates the eggs. During the day, the female is faced with a trade-off between spending time on the nest to maintain egg temperature and spending time away from the nest for self-maintenance (i.e. foraging). This trade-off, and thereby its consequences, is likely more pronounced in small passerines, since they cannot store large amount of energy and thus need to forage daily to balance their expenditure.

## METHODS

### Study population
This study was conducted in the woodlots of the Lauwersmeer, in the northern Netherlands (53º20' N – 6º12' E) during the breeding season of 2004. The woodlots consist of mixed deciduous tree species and contain about 200 nest-boxes. For further details see Tinbergen (2005). In this study population, clutches contain on average 9.3 ± (SD) 1.8 eggs (n = 1140; 1994-2003). Females may produce a second clutch after successfully raising a first clutch.

## General procedure

From the beginning of April, all nest-boxes were checked at least once a week to determine laying date and clutch size. Laying date was estimated by counting back from the observed clutch size, thereby, assuming that one egg was laid per day. The onset of incubation was determined by daily nests visits from the seventh egg onwards. Onset of incubation was defined as the first day the female was found incubating or the first day the eggs were found uncovered and warm.

## Experimental set-up

### Clutch size manipulation

From the available nests, 35 nests were randomly assigned to two treatment levels. Clutches were either kept as control (n = 19), and these birds were incubating their own clutch. Alternatively, clutches were experimentally enlarged (n = 16). These clutches were manipulated on average 3 ± 1.5 days before the measurement of energy expenditure and were enlarged by adding three eggs from donor nests which had a similar incubation stage. With this manipulation size, we adhere to previous studies on brood size manipulations in tit species (e.g. Sanz & Tinbergen 1999; Wiersma & Tinbergen 2003) to facilitate comparison. To prevent nest desertion of donor nests in response to clutch reduction, temporarily removed eggs were replaced by dummy eggs. The day after the energy measurement, eggs were returned to their nest of origin.

### Measurements of daily energy expenditure

Daily energy expenditure during incubation was measured using the doubly labelled water (DLW) technique (Lifson & McClintock 1966). On day 9 or 10 of incubation, i.e. three-quarter through the incubation period, females were caught with a hand-net on leaving the nest-box during the day (mean time 13:51 h; range 9:01- 16:13 h). Birds caught were identified, or ringed, and weighed. They were injected intraperitoneally with 0.10 ml of a mixture containing 5.4937 gram of 94.20 AP (% atom) $H_2^{18}O$ and 3.3606 gram of 99.90 AP (% atom) $D_2O$ using 0.3 ml insulin syringes (the exact dose per animal was measured by weighing syringes before and after injection). Daily energy expenditure was measured following one of the two protocols; the two-sample and the single sample protocol (Williams & Dwinnel 1990). Thirty females were measured with the single-sample protocol to minimize disturbance. These birds were released immediately after injection, and captured a second time after 25 hours to take a blood sample from the brachial vein in the wing. At taking this blood sample, individuals were weighed for the second time. Of five females, the daily energy expenditure was measured following the two-sample protocol to determine equilibrium values of DLW. These birds were kept in a cloth bag for an hour after injection to allow for equilibration of DLW with the bird's body water pool (Speakman *et al.* 2001). After this hour an initial blood sample was taken from the brachial vein of one of the wings. After that, the

bird was released. Twenty-four hours after release, and thus 25 hours after injection, birds were recaptured and a second and final blood sample was taken from the brachial vein in the other wing. Blood samples of four randomly selected, non-experimental females were taken at day 9 or 10 of their incubation period to determine background values of deuterium and oxygen-18 of the population. All blood samples were collected in triplets (20 $\mu$l; in total 60 $\mu$l) in heparinised glass capillaries that were flame-sealed immediately after sampling. After transport to the laboratory, samples were stored at room temperature in the dark.

Blood samples were analysed for deuterium and oxygen-18 concentration using isotope ratio mass spectrometry at the Centre for Isotope Research of the University of Groningen, the Netherlands. Mean background isotope enrichments of deuterium and oxygen-18 for non-experimental females were -19.74 $\pm$ 5.1 ‰ and -0.79 $\pm$ 0.47 ‰ relative to standard mean ocean water (V-SMOW). Daily $CO_2$ production was calculated using equation 35 of Lifson & McClintock (1966), assuming the body water pool to be on average 66% of the total body mass (data for great tit, Mertens 1987). For the animals in which the single sample protocol was applied, the isotope enrichment in the body water pool one hour after injection was calculated using the dose, average background concentrations, and an average body water pool of 66%. Subsequently, the same equation of Lifson & McClintock (1966) was applied (see also Speakman 1997). Daily energy expenditure ($DEE_{inc}$; kJ day$^{-1}$) was calculated from the $CO_2$ production using a respiratory quotient (RQ) of 0.75 and an energetic equivalent of 19.9 kJ l$^{-1}$ of oxygen consumed (following Tinbergen & Dietz 1994).

### Additional measurements
*Incubation behaviour*
Nest temperature was registered every 15 s by a sensor that was mounted between the eggs (HOBO logger, Mulder-Hardenberg B.V., The Netherlands). The behaviour of the incubating females was inferred on the basis of changes in nest temperature: a sudden drop in temperature represents the departure of the female from the nest, and a sharp increase the return of the female to the nest. For eight females, the measurements of incubation behaviour derived from temperature loggers were compared with those estimated from video recordings (mean total observation time 2h 22min). The time a female spent on the nest derived from the temperature loggers was highly correlated with that estimated from video ($R^2$ = 0.98).

The following times were recorded: the *length of the night* (s) defined as the time span between the last entry in the evening and the first exit in the morning, and the *length of the active day* (s) defined as the time span between the first exit in the morning and the last entry in the evening. During the active day, the following behaviours were scored: *latency to return* (s) defined as the time between release of the female after injection and first return of the female to the nest, *time*

*away from the nest* (s) and *time on the nest* (s) defined as the total time during the day that the female spent away from the nest (excluding the latency to return) and on the nest, *the number of recesses* (#) defined as the number of times the female went away from and returned to the nest during the day.

*Ambient temperature*
At a central location in the study area, a temperature sensor (Tinytalk II, Gemini Data Loggers, INTAB Benelux) recorded ambient temperatures (ºC) at 10 min interval at a height comparable to that of the nest-boxes. For each individual, mean ambient temperature was calculated for the entire 24-h period of measurement, as well as for the night and the day separately.

*Nest thickness*
During the incubation period, the thickness of the nest cup (i.e. the distance between the bottom of the nest cup and the bottom of the nest-box) was recorded one during incubation. Nest thickness was previously found to be a reliable measure of nest insulation (chapter 5), and to be a factor that reduced the metabolic rate of nocturnal incubation (chapter 6).

**Statistical analysis**
Of the 35 birds that were initially injected with DLW, 6 cases were excluded: 3 birds abandoned their nests after the first catch, 2 birds could not be caught in time for the final blood sample and for 1 bird, the $CO_2$ production could not be determined accurately. Therefore, the actual sample size was 29 (control, n = 14; enlarged, n = 15). For 4 of the 29 individuals, the incubation behaviour could not be determined due to malfunctioning of temperature loggers, which reduced the sample size to 25 individuals when analysing the incubation behaviour of the birds.

Data were analysed using GLM's in SPSS 12 for Windows. Effects of clutch size manipulations were studied on daily energy expenditure ($DEE_{inc}$), change in body mass (difference in body mass between time of injection and the final blood sample), the length of the night, latency to return, the number of recesses and the time spent on the nest during the active day. Hereby, the experimental treatment was treated as fixed factor and mean ambient temperature (ºC) as covariate. In addition, regression analyses were performed to study the effect of the experimental treatment and ambient temperature relative to that of original clutch size (#), nest thickness (mm) and body mass (gram). Interactions were tested, but not reported unless significant. All values are presented as means ± SD, unless stated otherwise.

## RESULTS

### Energy expenditure

Females in the two treatment categories did not differ in laying date (April date, control $17.4 \pm 6.9$, enlarged $17.3 \pm 6.4$; $F_{1,28} = 0.0$, $p = 0.97$), original clutch size (control $9.1 \pm 1.2$, enlarged $9.0 \pm 2.0$; $F_{1,28} = 0.1$, $p = 0.82$) and nest thickness before the experiment (in mm; control $11.4 \pm 7.9$, enlarged $11.3 \pm 6.1$; $F_{1,28} = 0.0$, $p = 0.95$). Also, body mass of the females at injection did not differ between the two treatment categories (in gram; control $20.1 \pm 1.0$, enlarged $19.7 \pm 1.1$; $F_{1,28} = 1.3$, $p = 0.26$). Over a 24-h period, females incubating enlarged clutches spent on average $79.1 \pm 9.6$ ($DEE_{inc}$, kJ day$^{-1}$), while those incubating control clutches spent $78.4 \pm 9.0$. $DEE_{inc}$ was strongly related to ambient temperature (Fig. 7.1 and Table 7.2). When controlled for ambient temperature, females incubating enlarged clutches did not expend more energy than females incubating control clutches (residual of the regression of $DEE_{inc}$ and ambient temperature, control $0.8 \pm 5.8$, enlarged $-0.7 \pm 8.4$; Table 7.2). Original clutch size, nest thickness and body mass did not explain any of the variation in $DEE_{inc}$ (Table 7.2).

### Utilisation of body reserves

During the DLW measurements of energy expenditure, birds experienced a slight change in body mass. On average, masses changed from $19.89 \pm 1.04$ gram at the time of injection to $19.72 \pm 1.03$ after 25 h period. Thus, birds lost on average $0.17 \pm 0.48$ gram; however, this loss was not statistically different from zero ($t_{1,28} = -1.92$, $p = 0.07$). Mass change did not differ between females of the two experimental groups, nor was it related to any other covariate (Table 7.2).
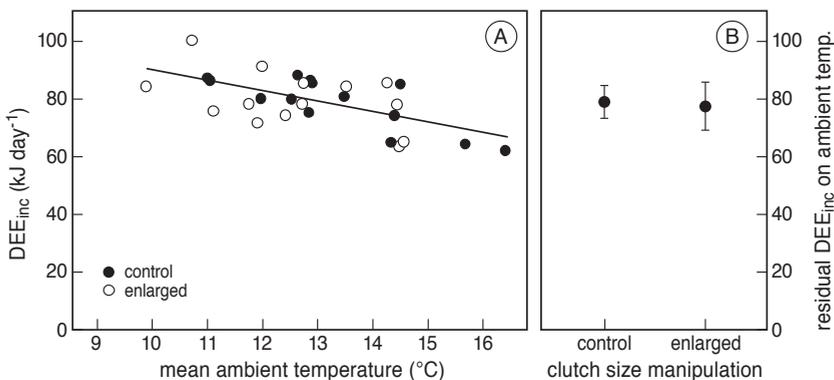


**Figure 7.1.** A) Daily energy expenditure during incubation ($DEE_{inc}$ kJ day$^{-1}$) of female great tits in relation to mean ambient temperature measured over the 24-h period for control and experimentally enlarged clutches. B) Residual $DEE_{inc}$ on ambient temperature for females incubating either enlarged or control clutches; values are added to the mean $DEE_{inc}$ of females in the control category.

**Table 7.2.** Test results for the effect of clutch size manipulations and several covariates on $DEE_{inc}$ and other response variables. Effect sizes (β) are only given for factors that had a significant effect. Asterisks indicate the temperature used, being either the entire 24-h period (no asterisks) or the night (*) or day (**) only.

| A. ANCOVA | $DEE_{inc}$ kJ day$^{-1}$ | | | Length of night (s) | | | BM change(g) | | Fraction latency to return | | Fraction time on nest | | | Number of recesses | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β±SE | F | p | β±SE | F | p | F | p | F | p | β±SE | F | p | F | p |
| Intercept | 127.2±11.8 | 113.6 | 0.001 | 32,173±2,137 | 220.0 | 0.001 | 0.6 | 0.45 | 2.9 | 0.10 | 0.6±0.2 | 11.9 | 0.001 | 2.9 | 0.10 |
| Mean ambient temperature | -3.8±0.9 | 17.0 | 0.001 | *432±204 | 4.5 | 0.05 | 1.0 | 0.34 | **0.2 | 0.62 | **0.3 | 0.60 | **1.1 | 0.31 |
| Clutch size manipulation | | 0.3 | 0.58 | | 1.2 | 0.28 | 0.3 | 0.60 | 0.0 | 0.90 | | 0.6 | 0.45 | 0.1 | 0.78 |

| B. REGRESSION ANALYSIS | $DEE_{inc}$ kJ day$^{-1}$ | | | Length of night (s) | | | BM change(g) | | Fraction latency to return | | Fraction time on nest | | | Number of recesses | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β±SE | t | p | β±SE | t | p | t | p | t | p | β±SE | t | p | t | p |
| Intercept | 126.2±11.6 | 10.2 | 0.001 | 31,998±2,142 | 14.9 | 0.001 | -1.9 | 0.07 | 6.5 | 0.001 | 0.6±0.2 | 30.8 | 0.001 | 20.9 | 0.10 |
| Mean ambient temperature | -3.7±0.9 | -4.1 | 0.001 | *488±199 | 2.5 | 0.02 | 0.9 | 0.37 | **-0.5 | 0.65 | **-0.5 | 0.59 | **1.1 | 0.31 |
| Clutch size manipulation | | -0.6 | 0.58 | | -1.1 | 0.28 | -0.3 | 0.73 | -0.0 | 1.00 | | 0.8 | 0.41 | -0.3 | 0.78 |
| Original clutch size | | -1.5 | 0.15 | | -0.6 | 0.56 | 0.9 | 0.38 | -0.2 | 0.83 | | 0.8 | 0.45 | -0.2 | 0.85 |
| Body mass | | 0.2 | 0.88 | | -1.3 | 0.20 | -0.2 | 0.87 | -1.1 | 0.29 | | 0.8 | 0.41 | -0.0 | 0.98 |
| Nest thickness | | -1.4 | 0.18 | | 0.1 | 0.95 | 0.0 | 0.98 | -1.4 | 0.17 | | 1.3 | 0.22 | 0.8 | 0.94 |

**Nest attendance**

Females spent on average 10h 20min ± 34 min during the night. The time females spent in the nest-box at night was not affected by clutch size manipulation, but was positively associated with mean ambient temperature during the night (Table 7.2): females spent shorter period in the nest-box on colder nights. This association could not be explained by change in day length due to date ($t_{1,24}$ = 0.30, P = 0.77).

After injection, females' latency to return was on average 2 h 29 min ± 1h 51 min. The remainder of the day, females alternated periods spent on the nest (on average 23.4 ± 8.4 min) with periods away from the nest (7.8 ± 2.0 min); on average they had 23.1 ± 5.5 recesses. In total, females spent 8h 24min ± 1h 29min on the nest and 3h 01min ± 51min away from the nest. Neither of these behaviours was affected by the experimental treatment or any of the other covariates (Table 7.2).

## DISCUSSION

Ambient temperature strongly influenced daily energy expenditure ($DEE_{inc}$, kJ $day^{-1}$). No difference in $DEE_{inc}$ of females incubating experimentally enlarged clutches compared to that of females incubating control clutches could be detected, when controlled for ambient temperature. Body masses of incubating females did not decline significantly in the course of the 24 h measuring period and did not differ between treatment categories, indicating that birds were in balance and did not use body reserves to cover their expenditure. Thus, female great tits did not respond to clutch enlargement by increasing their energy expenditure. These results are in agreement with the findings of Moreno and Carlson 1989 and Engstrand *et al*. 2002, but in contrast to those of Moreno *et al.* (1991) and Moreno and Sanz (1994).

We would have expected females incubating enlarged clutches to have higher energy expenditure than those incubating control clutches when measuring over 24-h for the following reason. Females incubating experimentally enlarged clutches are found to spend energy at a higher rate during nocturnal incubation (Biebach 1981; Biebach 1984; Haftorn & Reinertsen 1985; Weathers 1985), and this applies to our great tits as well (chapter 6). Over 24-h, females not only have to incubate the clutch, but also need to leave the clutch unattended to forage for self-maintenance and subsequently, need to rewarm the clutch upon return. Costs of rewarming the clutch are thought to be energetically more demanding than maintaining egg temperature (Vleck 1981; Biebach 1986), and may be clutch size related (Mertens 1977; Walsberg & King 1978, but see Biebach 1986). Yet, two options exist that may explain why in this study females of the two treatment categories did not differ in their daily energy expenditure. First, sample size may be

limited to have enough power to detect a significant difference in $DEE_{inc}$ between females of the two treatment categories. Alternatively, females may have been reluctant to increase their daily energy expenditure in reaction to clutch enlargement, and instead may have compensated behaviourally or physiologically.

To calculate the sample size needed to reveal a significant difference in $DEE_{inc}$ between females of the two treatment categories, data of a previous study are used (chapter 6). In this study, which has been performed in the same population, measurements on energy expenditure during nocturnal incubation in relation to clutch size reveal that females incubating enlarged clutches spend between 0.039 and 0.054 J s$^{-1}$ extra energy when incubating three additional eggs (chapter 6). If females incubating enlarged clutches are assumed to spend the same amount of extra energy over the full 24-h period when incubating three additional eggs – this would be equivalent to 3.4 - 4.7 kJ per day, the sample size needed to detect such a difference in $DEE_{inc}$ between females of the two treatment categories given the variance in DLW measurements would have been *two to four times* the current sample size (using power analysis in STATISTICA 7; t-test independent sample, using residuals of $DEE_{inc}$ on ambient temperature). Only if the clutch size related energetic costs of incubation during the day would have been larger than during the night, an effect of clutch enlargement would have been detected with the current sample size. We conclude that the effects of incubating enlarged clutches are too small to be detected with the DLW technique. This could be the result of large individual variation in behaviour or variation in measuring value (5 - 10% ; Tatner & Bryant 1989; Speakman 1997). These results question the importance of the level of energetic expenditure during the incubation phase as a causal mechanism behind selection on clutch size in avian life history. However, females may have compensated for the additional energetic demands with clutch enlargement, and hence kept similar $DEE_{inc}$.

Clutch size manipulations performed during the incubation period in the same population of nest-box breeding great tit revealed fitness costs of incubation (chapter 2 and 3). Eggs incubated in enlarged clutches have longer developmental time as compared to that of eggs incubated in either control or reduced clutches (chapter 3). Additionally, females that incubated enlarged first clutches, fledged fewer offspring in the subsequent breeding attempt in the same year (chapter 2). Moreover, these females had lower local survival probability (chapter 2). Also, other studies show fitness costs for females incubating enlarged clutches (Visser & Lessells 2001; Hanssen *et al.* 2005; chapter 2). The fitness costs for females incubating enlarged clutches imply that there are clutch size related costs involved. That we did not find an increase in energy expenditure can be because the costs are unrelated to energy expenditure or that females compensate in reaction to clutch enlargement. Moreno and Sanz (1994) show convincingly (with even smaller sample size; see Table 7.1) that female pied flycatchers spent less energy (on average 9.5 kJ per day per egg) when incubating experimentally reduced

clutches, but that they did not increase their expenditure with clutch enlargement. Given this finding by them and the clutch size related energetic costs at night, females seem most likely to be either reluctant or unable to increase their daily energy expenditure in reaction to clutch enlargement, and need to compensate.

Females may compensate for the additional energetic demands by changing their behaviour. Reid *et al.* (2000b) suggest that birds may change their nest attentiveness in reaction to clutch enlargement, as with clutch enlargement also the thermal property of the clutch changes. The fact that several studies report prolonged incubation (Moreno & Carlson 1989; Smith 1989; Siikamäki 1995; chapter 3) or reduced hatching probability (Moreno *et al.* 1991; Siikamäki 1995; Reid *et al.* 2000b; Engstrand & Bryant 2002) for offspring of enlarged clutches, indeed points in the direction that birds change their incubation behaviour. We, however, do not find any indication that females of the two categories behave differently, neither do Moreno *et al.* (1991) and Engstrand *et al.* (2004; Table 1). Nevertheless, we cannot rule out all possible strategies of behavioural compensation; females could incubate less intensively or change their behaviour in the time away from the nest. For instance, they could eat less preferred, but easier available prey or accept greater risk when foraging (Lima & Dill 1990). Given that in a previous study on great tits in the same population, eggs incubated in enlarged clutches had prolonged incubation hints in the direction of the first option.

Instead of compensating behaviourally, females may compensate by reallocating their energy from costly maintenance processes to other activities. Several studies determine the effects of working hard on physiology of individuals and found that individuals indeed reallocate energy from somatic maintenance (i.e. immunological defence systems or in DNA repair systems) to save energy (Deerenberg *et al.* 1997; Wiersma & Verhulst 2005). Studies that enlarged clutches during incubation report reduced defence against parasites and reduced immune competence for incubating females (Siikamäki *et al.* 1997; Hanssen *et al.* 2005). Reduced investment in the maintenance could explain the reduction in survival or future reproduction for parental birds (Hanssen *et al.* 2005).

In conclusion, daily energy expenditure of females incubating enlarged clutches was not substantially higher than that of females incubating control clutches. Due to the sample size, power is lacking to reveal any small difference in daily energy expenditure of female of the two treatment categories. Nevertheless, the fact that clutch size manipulations for the duration of the total incubation period in the same study population reveal fitness costs of incubation suggest that females most likely compensate for these costs by neglecting some (unmeasured) energetic maintenance functions. The mechanism by which these female compensate the additional energetic demand involved with clutch enlargement remains unclear, and needs to be the focus of future studies.

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