Metabolic rate of nocturnal incubation in female great tits, *Parus major*, in relation to clutch size measured in a natural environment

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**Summary**

To study the energetic costs of incubation in relation to clutch size, clutch sizes were manipulated and the metabolic rate of female great tits, *Parus major* (Linnaeus), during nocturnal incubation (MR\textsubscript{inc}) was measured using mobile oxygen analysers. Individuals were measured on consecutive nights while incubating their own or manipulated clutches. The experiment was performed under field conditions in order to place possible effects of clutch size manipulation within the context of other factors explaining variation in MR\textsubscript{inc}. Females spent more energy when incubating enlarged clutches as compared with controls (6–10% more energy for three additional eggs) but did not spend significantly less energy when incubating reduced clutches. MR\textsubscript{inc} was strongly negatively related to ambient temperature. The effect of clutch enlargement is consistent with previous studies whereas the absence of an effect of clutch reduction is not. The small effect of clutch enlargement on MR\textsubscript{inc} highlights the need for further studies to include measurements of daily energy expenditure in order to judge how important energy expenditure can be in explaining fitness consequences of incubating experimentally enlarged clutches.

Key words: clutch size manipulation, energy expenditure, natural selection, oxygen consumption.

**Introduction**

In birds, the annual peak of energy demand has long been thought to occur when parents provision their offspring with food during the nestling phase. This has lead to the idea that selection on clutch size takes place during the nestling phase (Lack, 1947; Drent and Daan, 1980; Walsberg, 1983; Daan et al., 1990), resulting in a wide range of studies on limits to parental care during this period (Lindén and Møller, 1989; Dijkstra et al., 1990; Vanderwerf, 1992). As a consequence, the energetic demands during other reproductive phases – such as egg laying and incubation – have long been ignored (Williams, 1996; Monaghan and Nager, 1997).

During incubation, avian eggs need external heat provisioning, regular turning and favourable humidity for proper embryonic development (Deeming, 2002); care that is often provided by one or both of the parents. The energetic costs of providing heat to the eggs have long been thought to be negligible. Increasing evidence suggests, however, that below thermo-neutrality the metabolic rate (energy spent per time unit) of an incubating female is higher than that of a non-incubating female at rest (Williams, 1996; Thomson et al., 1998; Tinbergen and Williams, 2002). Since temperatures are normally below thermo-neutrality at temperate latitudes, energetic costs of incubation may substantially add to the overall daily energy expenditure of attending parents.

It is now known that, over 24 h, energy expenditure in the incubation phase is of the same order of magnitude as expenditure in the nestling phase for a number of small passerines (Williams, 1996; Tinbergen and Williams, 2002). Whether these costs are related to clutch size remains to be investigated. The most accurate way to measure this is through the use of respirometry. For species breeding in nest-boxes, this technique can be applied in the field to determine the energetic costs of nocturnal incubation, because at night the attending parent remains in the nest-box. To put possible effects of clutch size variation within the context of other factors influencing the energy expenditure of incubating birds, measurements should preferably be determined in free-living individuals under field conditions.

Several studies have measured metabolic rate during nocturnal incubation in relation to clutch size (Biebach, 1981; Biebach, 1984; Haftorn and Reinertsen, 1985; Weathers, 1985). Of these studies, only the study by Haftorn and Reinertsen (Haftorn and Reinertsen, 1985) was under field conditions. All of these studies support the idea that clutch size manipulation affects metabolic rate during nocturnal incubation.

In the present study, we measured metabolic rate during nocturnal incubation (MR\textsubscript{inc}; J s\textsuperscript{-1}) in free-living female great tits, *Parus major*, using mobile oxygen analysers. We
attempted to establish the causal relationship between clutch size and nocturnal energy expenditure by repeatedly measuring the same individual experiencing different clutch size manipulations. We performed the measurements during first clutches in one year and second clutches in another year. The natural variation in ambient temperature experienced by the incubating birds during the two experiments enabled us to estimate the temperature dependence of energy expenditure during nocturnal incubation. In one of the two years, we additionally measured nest thickness and body mass. These two variables may explain variation in the metabolic rate of nocturnal incubation.

Materials and methods

Study population

This study was conducted in a population of nest-box breeding great tits in the woodlots of the Lauwersmeer, in the north of The Netherlands (53°20' N, 06°12' E). About 200 nest-boxes were available in eight woodlots of different size (0.06–1.06 km²) interspersed with non-breeding habitat. In this study population of great tits, clutches contained, on average, 9.3±1.8 eggs (N=1140; 1994–2003).

General procedure

Nest-boxes were checked at least once a week from the beginning of April to estimate laying date assuming that one egg was laid per day. Onset of incubation was defined as the first day that the female was found incubating or that the eggs were uncovered and warm and was determined by daily nest visits from the seventh egg onwards during first clutches and from the third egg onwards during late clutches. Further weekly nest checks were made to determine the success of the nests. Females were caught for identification and measurement of individual characteristics, such as body mass, when nestlings were between 7 and 10 days old (day of hatching=0).

Measuring oxygen consumption

Experimental set-up

To study the energetic costs during nocturnal incubation in relation to clutch size, the same experiment was performed twice in two different years: 2001 and 2004. In 2001, the study area was closed during the first few weeks of the breeding season due to an outbreak of foot-and-mouth disease. Consequently, the experiment was performed during late clutches (June–July) that year. To increase the natural range of ambient temperatures that incubating females experienced during the measurements, the experiment was repeated during first clutches (April–May) in 2004. As a result, measurements were performed during different breeding attempts in different years. Consequently, variation in oxygen consumption could not be attributed to variation between years or variation between breeding attempts; we refer to this as ‘year effect’. The experiment was performed as early as possible during the incubation period because the oxygen consumption of embryos rises exponentially during the second half of incubation; prior to this it is negligible (Vleck et al., 1980; Prinzinger et al., 1995).

Individuals that participated in the experiment were randomly selected. No individual was used in both years. Each individual was measured on two or three consecutive nights, with the same oxygen analyser, while incubating either manipulated or original clutch sizes. Females were assumed to respond to the experimental treatment as if the resulting clutch size were a result of their own decision (Lessells, 1993). In total, the oxygen consumption of 30 individuals was measured: 10 individuals during late clutches (between 14 June and 10 July) in 2001 and 20 individuals during first clutches (between 19 April and 12 May) in 2004.

Clutch size manipulation

Clutch sizes were manipulated during daytime (around noon) prior to the night that the oxygen measurements were performed. In 2001, clutches (clutch size 7.0±0.7 eggs; range 6–8) experienced a sequence of three treatments involving reduction, enlargement or control (i.e. original clutch size laid by the female). The sequence of manipulations was randomised in such a way that the sequence was either ‘reduced – control – enlarged’ or ‘enlarged – control – reduced’. In 2004, clutches (clutch size 8.8±1.4 eggs; range 5–11) were only enlarged or kept constant: clutches were not reduced in order to prevent nest desertion (de Heij et al., 2006). In this year, the sequence of manipulations was randomised. Clutches were experimentally manipulated by the addition and removal of three eggs (about one-third of the original clutch). In this, we followed previous studies on brood size manipulations in the same population (Sanz and Timmergen, 1999; Wiersma and Timmergen, 2003). Clutches of six eggs were reduced by two eggs to prevent nest desertion. Eggs that were added to the clutch for enlargement came from donor nests with eggs at about the same incubation stage.

Oxygen measurements

The nest-box (inner size of nest-box 8.5×25×12 cm; total volume 2.6 litres) was converted into a metabolic chamber in the days before the measurements by making the nest-box airtight (Fig. 1). Five small holes (diameter 5 mm) in the bottom of the nest-box ensured that air entered the nest-box from below, passing the incubating female and leaving the nest-box via a tube from which air was drawn from the nest-box for analysis.

Two portable one-channel oxygen analysers (Servomex 570; Crawborough, UK: hereafter called unit A and B), both powered by 12 V car batteries, were used to measure oxygen consumption. Each measuring session started by installing the units at around 22.00 h, by which time the female had already been in the nest-box for about two hours. At that time, the entrance hole was closed with a rubber stopper and the nest-box was ventilated. After a one-hour equilibration period, the unit was calibrated using nitrogen gas (0% O₂) and dry ambient air (assumed to be 20.93% O₂). Air was drawn from the nest-
Flow) for unit A and B, respectively, in 2001, and at 20
air was drawn from the nest-box inflow of the nest-box (arrows underneath nest-box; c), while sample
in the entrance hole (b). Reference air was measured close to the rubber was inserted between nest-box and lid (a) and a cork was placed the thickness of the nest was determined by the thickness of the nest cup (e) and the height of the nest rim (f).

Additional measurements

Besides oxygen measurements, ambient temperature and incubation behaviour of the attending females were recorded. Female body mass and nest thickness were only measured in 2004.

Ambient temperature was recorded once every minute in the vicinity of the nest-box. The mean temperature over the measurement period was used in the analysis.

To determine the behaviour of an incubating female, a temperature sensor (HOBO logger; Mulder-Hardenberg b.v., Haarlem, The Netherlands) was placed between the eggs to register the temperature of the nest every 15 s. All but one bird incubated normally during the oxygen measurements (see later).

We estimated female body mass, since catching incubating females at night led to high rates of nest desertion (in a pilot study in 2002, nest desertion was 40%; N=10). Estimates were derived from measurements of body mass from females caught during late incubation in 2004 (N=35). Females were captured with a hand-net on leaving the nest-box during the day. Using this technique, only 9% of the birds abandoned their nests. Of this sample, 18 females were also involved in the present study.

Body mass during late incubation (Minc; mean ± s.d. 20.2±1.0 g) was highly related to that of the same females during the nestling phase (Mnest): Minc=3.15±5.63 (s.e.m.)+ 0.96·Mnest (r²=0.43, P<0.01, N=14). This calculation was used to estimate body mass during early incubation from body mass during the nestling phase.

Nest thickness was recorded once during the incubation period in 2004, using a knitting needle as the measuring tool. Both the height of the nest rim (drim; distance from nest rim to bottom of the nest-box) and the thickness of the nest cup (dcup; the distance between the bottom of the nest cup and the bottom of the nest-box) were recorded to the nearest mm (Fig. 1).
temperature; excluding this individual in the model did not
change the results.
All analyses were performed with a hierarchical linear
regression model in MLwiN version 2.02 (Rasbash et al., 2000)
to account for repeated measurements. All variables and their
two-way interactions were tested by backward elimination
from the model. Three different analyses were performed,
because of slightly different experimental procedures in the two
years. Firstly, we tested whether in 2001 clutch size
manipulation (reduced, control, enlarged) affected MRinc.
Original clutch size, date, ambient temperature and oxygen
analyzer (unit A or B) were included in the model covariates.
Secondly, we tested whether clutch size manipulation (control
and enlarged) affected MRinc in 2004 but controlled for
additional covariates: body mass and nest thickness. Thirdly,
data from both years were used to test consistency between
years.

All values are presented as means ± s.d., unless stated
otherwise.

Results

First experiment; late clutches in 2001
The metabolic rate during nocturnal incubation (MRinc) of
girl great tits incubating control clutches (mean clutch size
7.0±0.7 eggs) was 0.55±0.07 J s⁻¹ at ambient temperatures
of 14.5±2.4°C. The clutch size manipulation affected MRinc
(Table 1A). Females had higher MRinc when incubating
experimentally enlarged clutches as compared with control
clutches, but MRinc was not lower when females were
incubating reduced clutches (post-hoc analysis; Table 1A;
Fig. 2A). Mean ambient temperature explained part of the
variation in MRinc (Table 1A). MRinc increased significantly as
the night temperature (Tn; °C) decreased (Fig. 3). Other
covariates such as original clutch size, date and oxygen
analyzer did not explain part of the variation in MRinc.

Second experiment; first clutches in 2004
The metabolic rate during nocturnal incubation was
0.65±0.09 J s⁻¹ for females incubating control clutches that
contain on average 8.8±1.4 eggs and at ambient temperatures
of 9.3±2.4°C. Height of the nest rim and thickness of the nest
cup were strongly related: d_cup=38.26±4.36(s.e.m.)+1.37±
0.30(s.e.m.)r_cup (r²=0.56, P<0.00, N=19). Analyses of MRinc
were therefore performed with d_cup as covariate. Experimental
treatment, mean ambient temperature and thickness of the nest
cup, all explained part of the variation in MRinc (Table 1B).
Females had higher MRinc when incubating experimentally
enlarged clutches as compared with control clutches (Fig. 2B).
The effect of clutch size manipulation on MRinc was similar to
that in the experiment in 2001. MRinc was negatively related to
mean nocturnal temperature (Fig. 3) and thickness of the nest.
Variation in body mass did not explain variation in MRinc.

Combined results
The experimental treatment, ambient temperature and year
explained a significant part of the variation in MRinc (Table 1C).
The effect of clutch enlargement relative to the mean MRinc in
each of the two years (6–10%; three additional eggs) was
similar in both years (treatment × year; χ²=0.29, d.f.=1,
P=0.59; N=29). The effect of ambient temperature did not
differ for the two experiments (temperature × year; χ²=0.09,
d.f.=1, P=0.79; N=29). The effect of ambient temperature on
MRinc was strong (43–49% per 10°C; mean ± s.d. 11.1±3.4°C).
Year (or breeding attempt) explained an additional part of the
variation in MRinc. MRinc of females incubating late clutches in
the year 2001 was higher than that of females incubating first
clutches in 2004, when controlled for ambient temperature and
clutch size manipulation.

Table 1. The results of three hierarchical linear regression models of MRinc (J s⁻¹) in relation to experimental treatment and
several covariates

<table>
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<tbody>
<tr>
<td></td>
<td>β</td>
<td>x²</td>
<td>d.f.</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.903±0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>-0.027±0.004</td>
<td>15.9</td>
<td>1</td>
</tr>
<tr>
<td>Manipulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged</td>
<td>0.054±0.015</td>
<td>13.5</td>
<td>1</td>
</tr>
<tr>
<td>Reduced</td>
<td>-0.011±0.015</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest thickness</td>
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<td></td>
</tr>
<tr>
<td>Natural clutch size</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen analyser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td></td>
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</tbody>
</table>

β values are means ± s.e.m.
Asterisks (*) indicate whether the covariate was tested in the model and rejected; while minus signs (–) indicate that the covariate was not tested in the particular model.
Discussion

Clutch size manipulation

We found $MR_{inc}$ to be non-linearly related to clutch size. Clutch enlargement caused incubating females to expend more energy, but clutch reduction did not result in a lowering of energy expenditure. The increase in energy expenditure with increased clutch size is consistent with the results of previous studies (Biebach, 1981; Biebach, 1984). The absence of an effect of clutch reduction is surprising. Assuming that each egg requires a constant amount of energy, we expected $MR_{inc}$ to be linearly related to clutch size. Also, previous studies found an effect of clutch size reduction (Haftorn and Reinertsen, 1985; Weathers, 1985) and/or found $MR_{inc}$ to be linearly related to clutch size (Biebach, 1981; Biebach, 1984).

We think these results can be explained when the number of eggs that can be in direct contact with the brood patch is limited. This number is referred to as a ‘threshold clutch size’. Above such a threshold clutch size, eggs that are not in contact with the female’s brood patch are likely to cool (Mertens, 1977a). Consequently, incubating birds will repeatedly rearrange the eggs to rewarm the cooled eggs. Rewarming has been shown to be energetically more costly than maintaining eggs at incubation temperatures (Vleck, 1981; Biebach, 1986). This may potentially cause an increase in energetic costs when clutch size is above the threshold clutch size.

To explain the difference in relationship between $MR_{inc}$ and clutch size found in our study (non-linear) with that in previous studies (linear), we looked in more detail at previous studies (Table 2). Haftorn and Reinertsen (Haftorn and Reinertsen, 1985) measured $MR_{inc}$ of a female blue tit after a clutch reduction from 13 to eight eggs. Under the hypothesis about the threshold clutch size, the effect of clutch reduction will depend on the manipulated clutch size relative to the threshold clutch size. If the clutch size after manipulation is above the threshold, clutch reduction can be expected to lower energy expenditure, whereas if it is below, no such effect is to be expected. This explanation may be valid for the results of Haftorn and Reinertsen but not for those of Biebach (Biebach, 1984). He reported a linear relationship between $MR_{inc}$ and clutch size for female starlings at three different temperatures that were below thermo-neutrality. A closer look at the data, however, suggests that a non-linear effect of clutch size on

Fig. 2. Residual metabolic rate of nocturnal incubation ($MR_{inc}$; corrected for ambient temperature) of females incubating reduced, control or enlarged clutches in the year 2001 (A) and control and enlarged clutches in the year 2004 (B). The black circles represent mean values (± s.e.m.), while the grey lines represent repeated measurements of the same individual.

Fig. 3. The metabolic rate of nocturnal incubation ($MR_{inc}$) for females incubating control clutches in relation to ambient temperature ($T_a$) for two years (filled symbols, 2001; open symbols, 2004). The line shows the regression line for both years combined ($MR_{inc} = -0.0206 \times T_a + 0.8438; r^2=0.57$).

Fig. 4. Metabolic rate during nocturnal incubation in relation to clutch size for female starlings at three different temperatures. Redrawn from Biebach (Biebach, 1984). The quadratic lines have been added to the original graph.
**Table 2. Overview of studies on passerines that determine energy expenditure of nocturnal incubation (MR_{inc}) in relation to clutch size**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Species</th>
<th>Body mass (g)</th>
<th>Clutch size manipulation</th>
<th>Change in clutch size</th>
<th>Sample size</th>
<th>Effect (% of MR_{inc})</th>
<th>Effect per egg (% of MR_{inc})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>Canary (S. canaria)</td>
<td>20.8</td>
<td>Reduced b</td>
<td>–2/-3</td>
<td>3</td>
<td>3–15</td>
<td>1–5</td>
<td>(Weathers, 1985)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>European starling (S. vulgaris)</td>
<td>80</td>
<td>Continue a</td>
<td>1–8</td>
<td>2</td>
<td>3–5</td>
<td>3–5</td>
<td>(Biebach, 1981)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>European starling (S. vulgaris)</td>
<td>80</td>
<td>Continue a</td>
<td>1–8</td>
<td>8</td>
<td>3–5</td>
<td>3–5</td>
<td>(Biebach, 1984)</td>
</tr>
<tr>
<td>Field</td>
<td>Blue tit (P. caeruleus)</td>
<td>11.5</td>
<td>Reduced b</td>
<td>–5</td>
<td>1</td>
<td>18</td>
<td>4</td>
<td>(Haftorn and Reinertsen, 1985)</td>
</tr>
<tr>
<td>Field</td>
<td>Great tit (P. major)</td>
<td>20.2</td>
<td>Reduced b</td>
<td>–2/-3</td>
<td>9, 0 c</td>
<td>0 (NS)</td>
<td>0</td>
<td>Present study</td>
</tr>
</tbody>
</table>

\*Clutch sizes were manipulated in steps of one egg over a range from one to eight eggs.  
\*Clutch size manipulation relative to control.  
\*Sample size given separately for two years of measurement.

$\text{MR}_{\text{inc}}$ may also exist in his study (Fig. 4). We reanalysed the data of Biebach (Biebach, 1984), taking values from his fig. 1999; Weathers et al., 2002), ambient temperature was anlysis to test for a non-linear effect of clutch size, this term was significant (0°C, $F_{1,12}=4.98, P=0.05$) or approached significance (10°C, $F_{1,12}=3.73$, $P=0.07$; –10°C, $F_{1,12}=3.98$, $P=0.07$). This analysis makes it plausible that the non-linear effect of clutch size on $\text{MR}_{\text{inc}}$ may be a general phenomenon.

**Metabolic rate of nocturnal incubation**

Several variables, other than clutch enlargement, explained variation in $\text{MR}_{\text{inc}}$. We will briefly discuss each of them.

As in previous studies (Biebach, 1984; Bryan and Bryant, 1999; Weathers et al., 2002), ambient temperature was strongly related to $\text{MR}_{\text{inc}}$. Fig. 3 shows that a change in ambient temperature of 10°C, which is equal to a change in $\text{MR}_{\text{inc}}$ of 43–49%, is likely to occur during a breeding attempt. Although temperature variation will affect $\text{MR}_{\text{inc}}$ considerably, for the energetic consequences of the timing of reproduction the mean ambient temperature is of prime importance. A shift in timing of two weeks results in a change in mean ambient temperature of about 2°C (M.E.d.H., unpublished). This is equal to a change in $\text{MR}_{\text{inc}}$ of about 9%. The strong fluctuation in ambient temperature may nevertheless affect the peak demand in energy expenditure of parents during reproduction.

The change in $\text{MR}_{\text{inc}}$ with ambient temperature was similar in both years (i.e. the interaction term was non-significant). Controlling for ambient temperature, there was still a year effect on $\text{MR}_{\text{inc}}$. The direction of this effect was counterintuitive. When corrected for ambient temperature, females spent more energy during late clutches in 2004 than during first clutches in 2004. Several explanations may apply. Firstly, parents may incubate more intensively during late clutches as compared with first clutches to accelerate embryonic development and thereby shorten the incubation period (Smith, 1989). Secondly, nests may be proportionally thinner during late clutches than during first clutches, because having a thick nest may be disadvantageous later in the season due to the risk of hyperthermia of nestlings (Mertens, 1977b).

Thirdly, parents of late clutches are likely to be a selection of the population; they may be the ones that invest more during incubation. Fourthly, any year difference (for instance humidity) may be explanatory. All the above explanations are worthy of further study.

In the year we measured nest thickness, birds with thicker nests had lower energy expenditure. A probable explanation for this effect is that thicker nests were better insulated (Hoi et al., 1994; Szentirmai et al., 2005). Nest insulation is known to be of importance for the incubation behaviour of attending parents (Reid et al., 1999; Cresswell et al., 2003). The fact that not all birds build well-insulated nests suggests that there is a cost to thick nests (Hansell, 2000).

**Implications**

Our finding that females expend more energy during nocturnal incubation when incubating experimentally enlarged clutches is a first step towards the detection of a potential mechanism underlying negative selection on clutch size during the incubation phase. The finding is consistent with the survival cost associated with enlarged, but not reduced, clutches in this population (de Heij et al., 2006). Nevertheless, measurements on energy expenditure over a full 24 h are needed in order to judge how important energy expenditure can be in explaining fitness consequences of incubating experimentally enlarged clutches.

**List of abbreviations**

- $\text{M}_{\text{inc}}$ (g)  
  mean body mass of females during late incubation  
- $\text{M}_{\text{nest}}$ (g)  
  mean body mass of females during nestling phase  
- $\text{MR}_{\text{tot}}$ (J s$^{-1}$)  
  total metabolic rate during nocturnal incubation of incubating female and embryos  
- $\text{MR}_{\text{emb}}$ (J s$^{-1}$)  
  metabolic rate of embryos  
- $\text{MR}_{\text{inc}}$ (J s$^{-1}$)  
  metabolic rate during nocturnal incubation of incubating female  
- $d_{\text{cup}}$ (mm)  
  thickness of the nest cup  
- $d_{\text{rim}}$ (mm)  
  height of the nest rim

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References


