

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

Maaïke E. de Heij*, Alexandra J. van der Graaf, Dennis Hafner and Joost M. Tinbergen

Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

*Author for correspondence (e-mail: m.e.de.heij@rug.nl)

Accepted 13 March 2007

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_{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_{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_{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_{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 led 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_{inc} ; $J s^{-1}$) 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; Prinzing 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 Tinbergen, 1999; Wiersma and Tinbergen, 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; Crowborough, 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-

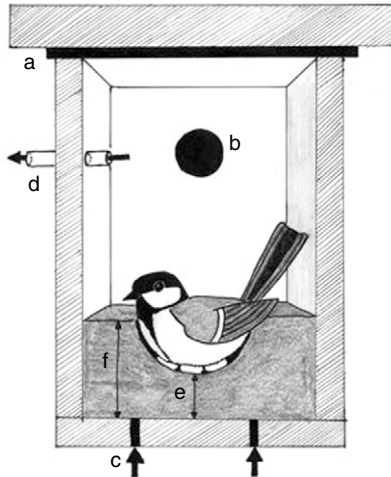


Fig. 1. Schematic drawing of a nest-box modified into a metabolic chamber. To ensure the top of the nest-box was airtight, a sheet of rubber was inserted between nest-box and lid (a) and a cork was placed in the entrance hole (b). Reference air was measured close to the inflow of the nest-box (arrows underneath nest-box; c), while sample air was drawn from the nest-box *via* a tube near the entrance hole (d). The thickness of the nest was determined by the thickness of the nest cup (e) and the height of the nest rim (f).

box and dried with molecular sieves 3\AA (Merck KgaA, Darmstadt, Germany) before flow measurements. Flow rates were kept constant at 20 and 15 l h^{-1} (10% of the maximum flow) for unit A and B, respectively, in 2001, and at 20 l h^{-1} for both units in 2004 with Brooks mass flow controllers (5860S; Brooks Instruments, Hatfield, PA, USA; accuracy $>99.0\%$). Air was sampled from an overflow and analysed in the oxygen analyser. During the measurement, ambient air (generally referred to as reference air) was sampled every hour for 10 min for unit A. For unit B, a valve automatically switched between sample air and reference air every 12 min in 2001 and every 24 min in 2004. Oxygen consumption was recorded for at least two hours in the period between midnight and 04.00 h. At the end of the measuring session, the unit was calibrated again. During nocturnal incubation, females maintained egg temperature throughout the entire night, although they regularly rose from the nest to turn eggs, to rearrange nest material or to resettle and face in a new direction (M.E.d.H., unpublished). Our measures of metabolic rate during nocturnal incubation included all these behaviours.

Oxygen consumption was calculated using equation 6 of Hill (Hill, 1972). The total metabolic rate of nocturnal incubation (MR_{tot}) included the metabolic rate of incubation by the incubating females (MR_{inc}) and that of the embryos (MR_{emb}). MR_{tot} was calculated assuming a respiratory quotient of 0.75 and an energy equivalent of 19.9 kJ l^{-1} oxygen consumed (Tinbergen and Dietz, 1994). Per sample period, data from at least the first three minutes were discarded to allow stabilising of the measurement. MR_{tot} was corrected for MR_{emb} . Knowing the incubation stage of the embryos of a clutch, MR_{emb} could be derived using data from Vleck et al. (Vleck et al., 1980). In

fig. 1c in their study, they summarised the relative MR_{emb} in relation to the relative incubation stage for altricial birds. By using the maximum MR_{emb} of great tits [J. A. L. Mertens, unpublished; cited in Vleck et al. (Vleck et al., 1980)], MR_{emb} could be derived for each egg at a particular incubation stage. Eggs from abandoned nests were assumed to be dead or only briefly incubated: their metabolic rate was not included in our calculations. MR_{inc} was calculated by subtracting the total MR_{emb} of a clutch from the MR_{tot} measured in the field; $MR_{\text{inc}} = MR_{\text{tot}} - MR_{\text{emb}}$. MR_{emb} was, on average, 0.010 J s^{-1} and 0.004 J s^{-1} per clutch in 2001 and 2004, respectively. Per night of measurement, mean values of MR_{inc} were used in the analysis.

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 (M_{inc} ; mean \pm s.d. $20.2 \pm 1.0\text{ g}$) was highly related to that of the same females during the nestling phase (M_{nest}): $M_{\text{inc}} = 3.15 \pm 5.63(\text{s.e.m.}) + 0.96 \cdot M_{\text{nest}}$ ($r^2 = 0.43$, $P < 0.01$, $N = 14$). 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 (d_{rim} ; distance from nest rim to bottom of the nest-box) and the thickness of the nest cup (d_{cup} ; the distance between the bottom of the nest cup and the bottom of the nest-box) were recorded to the nearest mm (Fig. 1).

Statistical analysis

Due to missing values, there is some variation in the sample size between analyses (in 2001, missing values for oxygen consumption = 2; in 2004, missing values for body mass = 2 and for nest thickness = 1). One bird did not continuously incubate the eggs, as judged from measurements of nest

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 MR_{inc} . Original clutch size, date, ambient temperature and oxygen analyser (unit A or B) were included in the model covariates. Secondly, we tested whether clutch size manipulation (control and enlarged) affected MR_{inc} 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 \pm s.d., unless stated otherwise.

Results

First experiment; late clutches in 2001

The metabolic rate during nocturnal incubation (MR_{inc}) of female great tits incubating control clutches (mean clutch size 7.0 ± 0.7 eggs) was $0.55 \pm 0.07 \text{ J s}^{-1}$ at ambient temperatures of $14.5 \pm 2.4^\circ\text{C}$. The clutch size manipulation affected MR_{inc} (Table 1A). Females had higher MR_{inc} when incubating experimentally enlarged clutches as compared with control clutches, but MR_{inc} 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 MR_{inc} (Table 1A). MR_{inc} increased significantly as the night temperature (T_a ; $^\circ\text{C}$) decreased (Fig. 3). Other covariates such as original clutch size, date and oxygen

analyser did not explain part of the variation in MR_{inc} .

Second experiment; first clutches in 2004

The metabolic rate during nocturnal incubation was $0.65 \pm 0.09 \text{ J s}^{-1}$ for females incubating control clutches that contain on average 8.8 ± 1.4 eggs and at ambient temperatures of $9.3 \pm 2.4^\circ\text{C}$. Height of the nest rim and thickness of the nest cup were strongly related: $d_{rim} = 38.26 \pm 4.36$ (s.e.m.) $+ 1.37 \pm 0.30$ (s.e.m.) $\cdot d_{cup}$ ($r^2 = 0.56$, $P < 0.00$, $N = 19$). Analyses of MR_{inc} 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 MR_{inc} (Table 1B). Females had higher MR_{inc} when incubating experimentally enlarged clutches as compared with control clutches (Fig. 2B). The effect of clutch size manipulation on MR_{inc} was similar to that in the experiment in 2001. MR_{inc} was negatively related to mean nocturnal temperature (Fig. 3) and thickness of the nest. Variation in body mass did not explain variation in MR_{inc} .

Combined results

The experimental treatment, ambient temperature and year explained a significant part of the variation in MR_{inc} (Table 1C). The effect of clutch enlargement relative to the mean MR_{inc} in each of the two years (6–10%; three additional eggs) was similar in both years (treatment \times year; $\chi^2 = 0.29$, d.f.=1, $P = 0.59$; $N = 29$). The effect of ambient temperature did not differ for the two experiments (temperature \times year; $\chi^2 = 0.09$, d.f.=1, $P = 0.79$; $N = 29$). The effect of ambient temperature on MR_{inc} was strong (43–49% per 10°C ; mean \pm s.d. $11.1 \pm 3.4^\circ\text{C}$). Year (or breeding attempt) explained an additional part of the variation in MR_{inc} . MR_{inc} 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 MR_{inc} (J s^{-1}) in relation to experimental treatment and several covariates

	A Experiment 1 (2001)				B Experiment 2 (2004)				C Combined results			
	β	χ^2	d.f.	P	β	χ^2	d.f.	P	β	χ^2	d.f.	P
Intercept	0.903 \pm 0.065				0.952 \pm 0.049				0.959 \pm 0.050			
Ambient temperature	-0.027 \pm 0.004	15.9	1	<0.001	-0.028 \pm 0.004	18.0	1	<0.001	-0.028 \pm 0.003	34.2	1	<0.001
Manipulation		14.1	2	<0.001								
Enlarged	0.054 \pm 0.015	13.5	1	<0.001	0.039 \pm 0.010	11.1	1	<0.001	0.044 \pm 0.007	22.5	1	<0.001
Reduced	-0.011 \pm 0.015	0.6	1	NS	–				–			
Year	–				–				-0.056 \pm 0.028	3.9	1	<0.001
Nest thickness	–				-0.004 \pm 0.002	3.7	1	0.05	–			
Natural clutch size	*				*				*			
Oxygen analyser	*				*				*			
Date	*				*				*			
Body mass	–				*				–			

β values are means \pm 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.

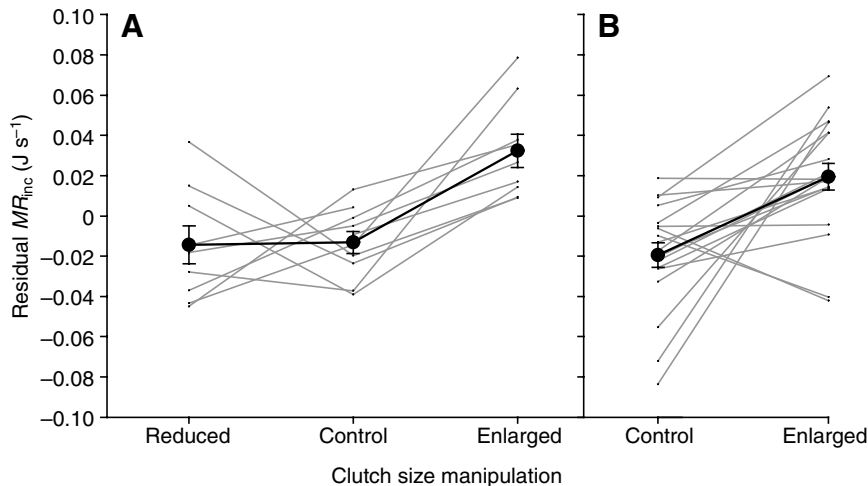


Fig. 2. Residual metabolic rate of nocturnal incubation (MR_{inc} ; corrected for ambient temperature) of females incubating reduced, control or enlarged clutches in the year 2001 (A) and control and enlarged clutches in the year 2004 (B). The black circles represent mean values (\pm s.e.m.), while the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

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).

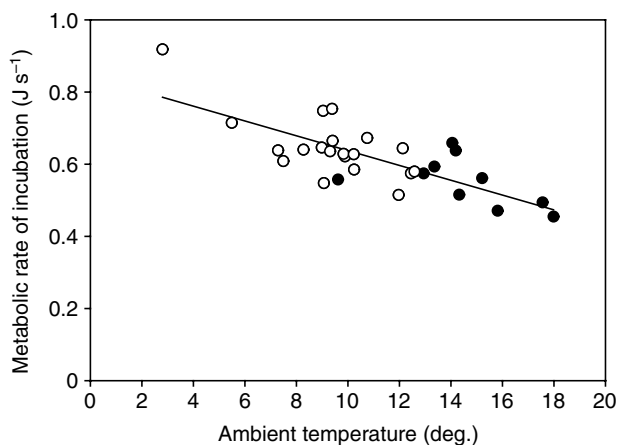


Fig. 3. The metabolic rate of nocturnal incubation (MR_{inc}) for females incubating control clutches in relation to ambient temperature (T_a) for two years (filled symbols, 2001; open symbols, 2004). The line shows the regression line for both years combined ($MR_{inc} = -0.0206 \times T_a + 0.8438$;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

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

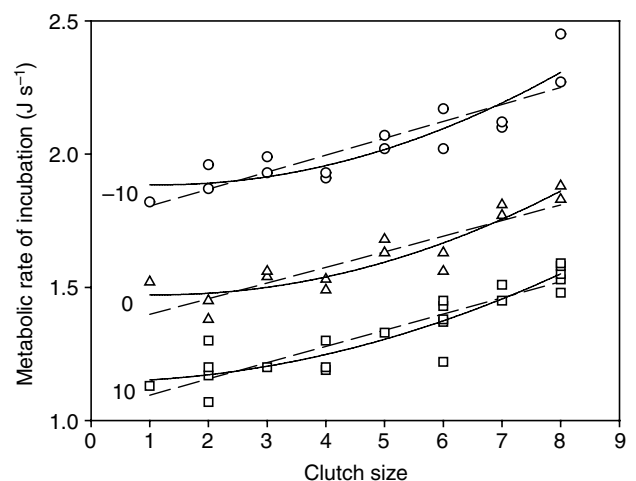


Fig. 4. Metabolic rate during nocturnal incubation in relation to clutch size for female starlings at three temperatures. Redrawn from Biebach (Biebach, 1984). The quadratic lines have been added to the original graph.

Table 2. Overview of studies on passerines that determine energy expenditure of nocturnal incubation (MR_{inc}) in relation to clutch size

Setting	Species	Body mass (g)	Clutch size manipulation	Change in clutch size	Sample size	Effect (% of MR_{inc})	Effect per egg (% of MR_{inc})	Reference
Laboratory	Canary (<i>Serinus canaria</i>)	20.8	Reduced ^b	-2/-3	3	3-15	1-5	(Weathers, 1985)
Laboratory	European starling (<i>Sturnus vulgaris</i>)	80	Continue ^a	1-8	2	3-5	3-5	(Biebach, 1981)
Laboratory	European starling (<i>Sturnus vulgaris</i>)	80	Continue ^a	1-8	8	3-5	3-5	(Biebach, 1984)
Field	Blue tit (<i>Parus caeruleus</i>)	11.5	Reduced ^b	-5	1	18	4	(Haftorn and Reinertsen, 1985)
Field	Great tit (<i>Parus major</i>)	20.2	Reduced ^b Enlarged ^b	-2/-3 3	9, 0 ^c 9, 20 ^c	0 (NS) 6-10	0 2-3	Present study

^aClutch sizes were manipulated in steps of one egg over a range from one to eight eggs.

^bClutch size manipulation relative to control.

^cSample size given separately for two years of measurement.

MR_{inc} may also exist in his study (Fig. 4). We reanalysed the data of Biebach (Biebach, 1984), taking values from his fig. 2. When in addition to clutch size a quadratic term was included in the analysis 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 , $F_{1,19}=3.73$, $P=0.07$; -10°C , $F_{1,12}=3.98$, $P=0.07$). This analysis makes it plausible that the non-linear effect of clutch size on MR_{inc} may be a general phenomenon.

Metabolic rate of nocturnal incubation

Several variables, other than clutch enlargement, explained variation in MR_{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 MR_{inc} . Fig. 3 shows that a change in ambient temperature of 10°C , which is equal to a change in MR_{inc} of 43-49%, is likely to occur during a breeding attempt. Although temperature variation will affect MR_{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 MR_{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 MR_{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 MR_{inc} . The direction of this effect was counterintuitive. When corrected for ambient temperature, females spent more energy during late clutches in 2001 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

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

We thank Allert Bijleveld, Maarten Bleeker, Kasper de Bruin, Douwe Schut, Julia Stahl, Richard Ubels and Wouter Vahl for their assistance in the field. The Royal Netherlands Army 'De Marne' and the Dutch Forestry Commission 'Lauwersmeer' kindly allowed us to work in their area. We thank the Royal Netherlands Army 'De Marne' for their hospitality and letting us stay at their barracks during fieldwork. We are grateful to Gerard Overkamp and Maurine Dietz for their technical support on the oxygen measurements and to Marcel Visser for valuable help and discussion about the oxygen measurements. We thank Rudi Drent, Wouter Vahl, Maurine Dietz, Theunis Piersma, Henk Visser, Nick Horrocks and two anonymous reviewers for commenting on earlier versions of this paper. The experiments comply with the current Dutch laws (DEC nr. 2685). Financial support came from the NWO-ALW (project nr. 810.43.009 to J.M.T.).

References

- Biebach, H.** (1981). Energetic costs of incubation on different clutch sizes in starlings (*Sturnus vulgaris*). *Ardea* **69**, 141-142.
- Biebach, H.** (1984). Effect of clutch size and time of day on the energy expenditure of incubating Starlings (*Sturnus vulgaris*). *Physiol. Zool.* **57**, 26-31.
- Biebach, H.** (1986). Energetics of rewarming a clutch in starlings (*Sturnus vulgaris*). *Physiol. Zool.* **59**, 69-75.
- Bryan, S. M. and Bryant, D. M.** (1999). Heating nest-boxes reveals an energetic constraint on incubation behaviour in great tits, *Parus major*. *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 157-162.
- Cresswell, W., Holt, S., Reid, J. M., Whitfield, D. P. and Mellanby, R. J.** (2003). Do energetic demands constrain incubation scheduling in a biparental species? *Behav. Ecol.* **14**, 97-102.
- Daan, S., Dijkstra, C. and Tinbergen, J. M.** (1990). Family planning in the kestrel (*Falco tinnunculus*): the ultimate control of variation in laying date and clutch size. *Behaviour* **114**, 83-116.
- de Heij, M. E., Van den Hout, P. J. and Tinbergen, J. M.** (2006). Fitness cost of incubation in great tits (*Parus major*) is related to clutch size. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 2353-2361.
- Deeming, D. C.** (2002). Behaviour patterns during incubation. In *Avian Incubation: Behaviour, Environment, and Evolution* (ed. D. C. Deeming), pp. 63-87. New York: Oxford University Press.
- Dijkstra, C., Bult, A., Bijlsma, S., Daan, S., Meijer, T. and Zijlstra, M.** (1990). Brood size manipulations in the kestrel (*Falco tinnunculus*): effects on offspring and parent survival. *J. Anim. Ecol.* **59**, 269-286.
- Drent, R. H. and Daan, S.** (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* **68**, 225-252.
- Haftorn, S. and Reinertsen, R. E.** (1985). The effect of temperature and clutch size on the energetic cost of incubation in a free-living blue tit (*Parus caeruleus*). *Auk* **102**, 470-478.
- Hansell, M.** (2000). *Bird Nests and Construction Behaviour*. Cambridge: Cambridge University Press.
- Hill, R. W.** (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. Appl. Physiol.* **33**, 261-263.
- Hoi, H., Schleicher, B. and Valera, F.** (1994). Female mate choice and nest desertion in penduline tits, *Remiz pendulinus*: the importance of nest quality. *Anim. Behav.* **48**, 743-746.
- Lack, D.** (1947). The significance of clutch size. *Ibis* **89**, 302-352.
- Lessells, C. M.** (1993). The cost of reproduction: do experimental manipulations measure the edge of the options set? *Etologia* **3**, 95-111.
- Lindén, M. and Møller, A. P.** (1989). Cost of reproduction and covariation of life history traits in birds. *Trends Ecol. Evol.* **4**, 367-371.
- Mertens, J. A. L.** (1977a). The energy requirements for incubation in great tits, *Parus major* L. *Ardea* **65**, 184-196.
- Mertens, J. A. L.** (1977b). Thermal conditions for successful breeding in great tits (*Parus major* L.). I. Relation of growth and development of temperature regulation in nestling great tits. *Oecologia* **28**, 1-29.
- Monaghan, P. and Nager, R. G.** (1997). Why don't birds lay more eggs. *Trends Ecol. Evol.* **12**, 270-274.
- Prinzinger, R., Schmidt, M. and Dietz, V.** (1995). Embryogeny of oxygen consumption in 13 altricial and precocial birds. *Respir. Physiol.* **100**, 283-287.
- Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I. and Lewis, T.** (2000). *A User's Guide to MLwiN* (2nd edn). London: Institute of Education.
- Reid, J. M., Monaghan, P. and Ruxton, G. D.** (1999). The effect of clutch cooling rate on starling, *Sturnus vulgaris*, incubation strategy. *Anim. Behav.* **58**, 1161-1167.
- Sanz, J. J. and Tinbergen, J. M.** (1999). Energy expenditure, nestling age, and brood size: an experimental study of parental behavior in the great tit *Parus major*. *Behav. Ecol.* **10**, 598-606.
- Smith, H. G.** (1989). Larger clutches take longer to incubate. *Ornis Scand.* **20**, 156-158.
- Szentirmai, I., Szekely, T. and Liker, A.** (2005). The influence of nest size on heat loss of penduline tit eggs. *Acta Zool. Acad. Sci. Hung.* **51**, 59-66.
- Thomson, D. L., Monaghan, P. and Furness, R. W.** (1998). The demands of incubation and avian clutch size. *Biol. Rev.* **73**, 293-304.
- Tinbergen, J. M. and Dietz, M. W.** (1994). Parental energy expenditure during brood rearing in the great tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. *Funct. Ecol.* **8**, 563-572.
- Tinbergen, J. M. and Williams, J. B.** (2002). Energetics of incubation. In *Avian Incubation: Behaviour, environment, and Evolution*. Vol. 13 (ed. D. C. Deeming), pp. 299-313. New York: Oxford University Press.
- Vanderwerf, E.** (1992). Lack's clutch size hypothesis: an examination of the evidence using meta-analysis. *Ecology* **73**, 1699-1705.
- Vleck, C. M.** (1981). Energetic cost of incubation in the Zebra Finch. *Condor* **83**, 229-237.
- Vleck, C. M., Vleck, D. and Hoyt, D. F.** (1980). Patterns of metabolism and growth in avian embryos. *Am. Zool.* **20**, 405-416.
- Walsberg, G. E.** (1983). Avian ecological energetics. In *Avian Biology* (ed. D. S. Farner, J. R. King and K. C. Parkes), pp. 161-220. New York: Academic Press.
- Weathers, W. W.** (1985). Energy cost of incubation in the canary. *Comp. Biochem. Physiol.* **81A**, 411-413.
- Weathers, W. W., Davidson, C. L., Olson, C. R., Morton, M. L., Nur, N. and Famula, T. R.** (2002). Altitudinal variation in parental energy expenditure by white-crowned sparrows. *J. Exp. Biol.* **205**, 2915-2924.
- Wiersma, P. and Tinbergen, J. M.** (2003). No nocturnal energetic savings in response to hard work in free-living great tits. *Neth. J. Zool.* **52**, 263-279.
- Williams, J. B.** (1996). Energetics of avian incubation. In *Avian Energetics and Nutritional Control* (ed. C. Carey), pp. 375-415. New York: Chapman and Hall.