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Costs of avian incubation

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

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

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ABSTRACT

To study clutch size related energetic costs of incubation, clutch sizes were experimentally manipulated and metabolic rate of female great tits *Parus major* (L.) during nocturnal incubation (MR_{inc}) was measured with the use of mobile oxygen analysers. Each individual was repeatedly measured, while incubating either reduced, control or enlarged clutches. In contrast to most previous studies, the experiment was performed in the field to put possible effects of the clutch size manipulation in perspective to other factors explaining variation in MR_{inc}. Females spent on average 0.65 ± 0.09 J s^{-1} during first clutches in 2004 and 0.55 ± 0.07 J s^{-1} during second clutches in 2001 at mean ambient temperatures of 9.3 ± 2.4 °C, and 14.5 ± 2.4 °C, respectively. Females spent energy at a higher rate when incubating enlarged clutches (6 - 10%; three additional eggs), but did not spend significantly less energy when incubating reduced clutches as compared to controls. The effect of clutch size on MR_{inc} was relatively small compared to that of ambient temperature, which explained most of the variation in MR_{inc} (43 - 49% per 10 °C). Nest thickness and year (or breeding attempt) explained an additional part of the variation. MR_{inc} exceeded that of basal or metabolic rate of non-incubating birds (2.0 times BMR, and $1.2 - 1.6$ times $MR_{8°C}$, respectively). Comparison between species reveals that MR_{inc} is relatively high for smaller bird species, which can be explained by the energetic costs of thermoregulation. The incubation phase may be a demanding period due to its timing in the season.

INTRODUCTION

In birds, the peak of energy demand has long been thought to occur during the nestling phase when parents need to provision their offspring with food, which lead to the idea that selection on clutch size would take place during this phase (Daan *et al.* 1990; Drent and Daan 1980; Lack 1947; Walsberg 1983). This stimulated a wide range of studies on limits to parental care during the nestling phase (Dijkstra *et al.* 1990; Lindén and Møller 1989; Vanderwerf 1992), but energetic demands during other reproductive phases have long been ignored (Monaghan and Nager 1997; Williams 1996).

During the incubation phase, avian eggs need external heat provisioning, regular turning and favourable humidity for proper embryonic development (Deeming 2002); this care is often provided by one or both of the parents. The energetic costs of providing heat to the eggs by the parent bird, called contact incubation, have long been subject of debate. The prevailing view, which was mainly based on biophysical models, has been that contact incubation was not energetically costly (King 1973; Walsberg and King 1978). King (1973) argued that the heat needed for incubation was provided by basal heat production, and that contact incubation would not require additional energy. In line with this view, Walsberg and King (1978) constructed a model that predicted energy expenditure during contact incubation to be greatly reduced by the insulation of the nest. Models by Kendeigh (1963) and Mertens (1977) had a different prediction, namely that energy expenditure of an incubating bird exceeds that of a non-incubating bird. Despite the empirical evidence provided by Biebach (1981), these models received little attention.

Accumulating data show, however, that the metabolic rate (energy spent per time unit) of an incubating female is higher than that of a non-incubating female at rest (Thomson *et al.* 1998; Tinbergen and Williams 2002; Williams 1996), at least when ambient temperatures are below thermoneutrality (temperatures at which individuals do not produce extra heat to maintain homoiothermy). At temperate latitudes, incubation generally takes place early in spring when ambient temperatures are below thermoneutrality, and thus well below temperatures favourable for embryonic development (Webb 1987). Moreover, the lower critical temperature, the lower boundary of the thermal neutral zone, is higher for incubating birds as compared to non-incubating individuals (Haftorn and Reinertsen 1985) These factors all suggest that contact incubation may be demanding for the attending parent.

To be of importance for selection on clutch size, the energetic costs of incubation need to be clutch size related. Under natural conditions, confounding effects may mask clutch size related costs and, therefore, such costs need to be studied by experimentally altering clutch sizes and measuring the consequences in terms of energy expenditure. These measurements are preferably determined in free-living individuals under field conditions to enable putting possible effects of clutch size

variation in perspective to other factors influencing the energy expenditure of incubating birds. Moreover, environmental conditions are generally fluctuating and unpredictable, and possibly influence the energy expenditure of free living individuals (Pendlebury *et al.* 2004). The energy expenditure during contact incubation can best be studied by use of respirometry given the accuracy of the measurements. Respirometry, nevertheless, requires air to be drawn from a closed system in which an individual is present, limiting this technique to long periods of relatively inactivity. Therefore, this technique can best be applied at night, when the parent is continuously present on the nest. Although several studies have measured the metabolic rate during nocturnal incubation in relation to clutch size (Biebach 1981; Biebach 1984; Haftorn and Reinertsen 1985; Weathers 1985), most of these studies did not use this technique under field conditions probably for logistic reasons (but see Haftorn and Reinertsen 1985).

To study the clutch size related energetic costs of contact incubation in free-living female great tits *Parus major* – a species with uniparental incubation – we manipulated clutch size and measured the metabolic rate during nocturnal incubation (MR_{inc}, J s^{-1}) using mobile oxygen analysers. In this way we combined the precision of respirometry with the natural conditions experienced by the incubating birds. By repeatedly measuring the same individual in the course of a clutch size manipulation we aimed at establishing the causal relation between clutch size and nocturnal energy expenditure. During the measurements, the incubating birds experienced a wide range of ambient temperatures through natural variation, which enabled us to estimate the temperature dependence of nocturnal energy expenditure as well. We measured both during first and second clutches to increase the range of ambient temperatures. Additionally, we measured nest thickness (as indicator of nest insulation Szentirmai *et al.* 2005; chapter 5) and body mass to possibly explain extra variation in the metabolic rate of nocturnal incubation. We compared the measured MR_{inc} with both calculated values of basal metabolic rate (BMR) and that of metabolic rate at rest measured at the same temperatures as during incubation (MR) to quantify the energetic costs of incubation. We also used these measures to compare the energetic costs of incubation for great tits with that of other species.

METHODS

Study population

This study was conducted in a population of nest-box breeding great tits *Parus major* in the woodlots of the Lauwersmeer, in the north of the Netherlands (53º20'N, 06º12'E). Since 1994, about 200 nest-boxes were available in 8 woodlots of different size (6 -106 ha) interspersed with non-breeding habitat. For details and a map of the area see Tinbergen (2005).

In this study population of great tits, clutches contained on average 9.3 eggs ± 1.8 (n = 1140; 1994-2003). Part of the females (9-51%; 1994-2003) produced a second clutch after successfully rearing a first clutch. During laying of the first clutches a female increases the fraction of the night spent incubating with each egg laid; full incubation starts after clutch completion (Haftorn 1988; pers. obs.). During late clutches, the female generally initiate the onset of incubation before clutch completion (pers. obs.). During nocturnal incubation the female maintains egg temperature throughout the entire night, though she regularly rises from the nest to turn eggs, to tremble-thrust the nest material and to re-settle and face a new direction (pers. obs.). The measure of the metabolic rate during nocturnal incubation included all these behaviours.

General procedure

Nest-boxes were checked at least once a week from the beginning of April to esti-mate the laying date assuming that one egg was laid per day. Onset of incubation was defined as the first day the female was found incubating or that the eggs were uncovered and warm, and was determined by daily nests visits from the seventh egg onwards during first clutches and from the third egg onwards during late clutches.

The experiment was performed as early as possible during the incubation peri-od (see below), because the oxygen consumption of embryos rises exponentially; the oxygen consumption of embryos is negligible till about 50% of the incubation period (Prinzinger *et al.* 1995; Vleck *et al.* 1980). Further weekly nests checks allowed for determining the success of the nests. Parents were caught for identifi-cation and measurement of individual characteristics, such as body mass, when nestlings were between 7- 10 days old (day of hatching = 0).

Measuring oxygen consumption

Experimental set-up

To study the clutch size related energetic costs during nocturnal incubation, two runs of the same experiment were performed in two different years; 2001 and 2004. In 2001, the study area was closed during the first few weeks to prevent further breakout of Foot and Mouth Disease. Consequently, the experiment was performed during second clutches (June – July) that year. To increase the natural range of ambient temperatures experienced during the measurements, the experi-ment was repeated with first clutches (April – May) in 2004. As a result, measure-ments were performed during different breeding attempts in different years, and variation in oxygen consumption due to differences between years or breeding attempts could not be separated. Individuals that participated in the experiment were randomly selected. Each individual was measured on two or three consecu-tive nights with the same oxygen analyser, while incubating manipulated clutch sizes. Besides measuring the oxygen consumption in relation to clutch size, meas-

urements of ambient temperature were taken in both years, while measurements of body mass and of nest thickness were taken in 2004 only. In total, the oxygen consumption of 30 individuals was measured (Table 6.1): 10 individuals during second clutches (between 14 June and 10 July) in 2001 and 20 individuals during first clutches (between 19 April and 12 May) in 2004.

Table 6.1. The number of individuals measured per manipulation category (with missing values in brackets) for two oxygen analysers (A and B) and two years/ breeding attempts (first clutches in 2001 and second clutches in 2004).

year	treatment	oxygen analyser		nr of individuals
		A	B	
2001	reduced	7	2 (1)	9
	control	7	3	10
	enlarged	7	2 (1)	9
2004	reduced	-	-	-
	control	11	9	20
	enlarged	11	9	20

Clutch size manipulation

Clutch sizes were manipulated around noon on the day that the metabolic rate during nocturnal incubation was measured. Clutch sizes were experimentally reduced or enlarged by three eggs (about 1/3 of the original clutch). In this, we followed previous studies on brood size manipulations in tit species that usually added three nestlings to the broods (e.g. Sanz and Tinbergen 1999; Wiersma and Tinbergen 2003). Clutches of six eggs, however, were reduced with two eggs to prevent clutches from becoming too small. Eggs that were added to the clutch for enlargement were coming from donor nests with eggs of about the same incubation stage. In 2001, clutches (clutch size; 7.0 ± 0.7, range 6-8) were reduced, enlarged or kept constant. 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, range 5-11) were either enlarged or kept constant in randomised order. Clutches were not reduced to prevent nest desertion (chapter 2).

Oxygen measurements

The nest-box (inner size of nest-box: 8.5· 25· 12 cm = 2.6 litre) was converted into a metabolic chamber in the days before the measurements by making the nest-box air tight (Fig. 6.1). Five small holes (Ø = 5 mm) in the bottom of the nest-box ensured that air was coming into the nest-box from below, passing the
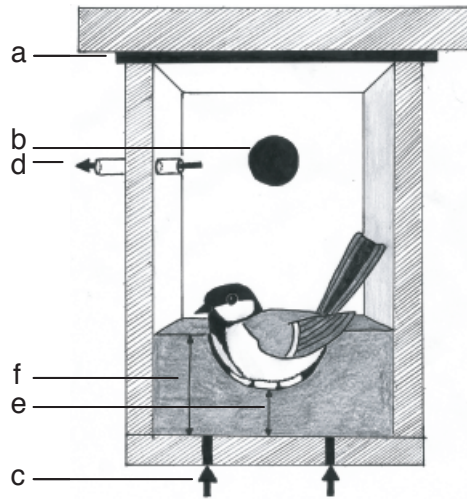
Figure 6.1. Schematic drawing of a nest-box modified into a metabolic chamber. To get the top of the nest-box airtight a mouse pad was inserted between nest-box and lid (a) and a cork was placed in the entrance hole (b). Reference air was measured close to the inflow of the nest-box (arrows underneath 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).

incubating female and leaving the nest-box via a tube from which air was drawn from the nest-box. A temperature sensor (HOBO logger, Mulder-Hardenberg B.V., The Netherlands) was placed between the eggs to register the temperature of the nest every 15 seconds from which the behaviour of the incubating female could be derived (all but one bird incubated normally; see later).

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. The protocol for both oxygen analysers was similar. In general, each measuring session started by installing the units around 22 h, when the female was already 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 one hour equilibration period, the unit was calibrated using nitrogen gas (0% O_2) and dry ambient air (assumed to be 20.93% O_2). Air was drawn from the nest-box, and dried with molecular sieves 3Å (Merck KgaA, Darmstadt, Germany) before flow measurements. In 2001, flow rates were kept constant at 20 and 15 l/hr (10 % of the maximum flow), for unit A and B respectively, with Brooks mass flow controllers (5860S, Brooks Instruments, Hatfield, USA; accuracy < 1.0%). In 2004, flow rates of both units were kept at 20 l/hr. 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 minutes for unit A. For unit B a valve automatically switched between sample air and reference air every 12 minutes in 2001 and every 24 minutes in 2004. Oxygen consumption was recorded for at least two hours in the period between midnight and 4 o'clock in the morning. At the end of the measuring session the unit was calibrated again.

Additional measurements
Ambient temperature
During the measurements, ambient temperature was recorded once every minute in the vicinity of the nest-box. Hence measurements were performed at the range of temperatures that birds experience during nocturnal incubation under field conditions. Per night of measurement, an average value of ambient temperature was used in the analysis.

Body mass
Preferably, individuals are weighed directly after measuring their metabolic rate to explain variation in oxygen consumption. In our case, we were not able to take females' body masses directly after the measurement, because taking incubating females from the nest at night generally leads to high rates of nest abandonment; 40% of birds (n=10) abandoned their nest (pers. obs.). In 2004, however, daily energy expenditure of 35 females was measured with the use of the doubly labelled water technique at three-quarters of the incubation period (chapter 7). These females were caught with a hand-net on leaving the nest-box during the day; 9% of the birds (n= 35) abandoned their nests after a first catch. Of this sample, 18 females were also involved in the present study. Mean body mass during late incubation (M_{inc}) of these 18 females was 20.2 ± 1.0 gram. Body mass during late incubation was highly correlated with that of the same females during the nestling phase ($M_{nestling}$): M_{inc} (gram) $= 0.96 \cdot M_{nestling}$ (gram) $+ 3.15$. The body mass during late incubation is assumed to approximate the body mass during early incubation, when the oxygen measurements were performed. Additionally, body masses measured during late incubation were used to approximate basal metabolic rate (BMR) of incubating females using the equation of (Kendeigh *et al.* 1977) for passerines (BMR (kJ day^{-1}) $= 0.8906 \cdot M$ (gram)$^{0.6884}$), which results in a predicted BMR level of 0.34 (J s^{-1}).

Nest thickness
In 2004, nest thickness was recorded once during the incubation period, using a knitting needle as measuring tool. Both the height of the nest rim (N_{rim}; distance from nest rim to bottom of the nest-box) and the thickness of the nest cup (N_{cup}; the distance between the bottom of the nest cup and the bottom of the nest-box) were recorded to the nearest mm (Fig. 6.1).

Calculations

Oxygen consumption was calculated using equation 6 of Hill (1972). The total metabolic rate of nocturnal incubation (MR_{total}) included the metabolic rate of incubation by the incubating females (MR_{inc}) and that of the embryos ($MR_{embryos}$). MR_{total} was calculated assuming a RQ of 0.75 and an energy equivalent of 19.9 kJ per litre oxygen consumed (following Tinbergen and Dietz 1994). Per sample period, data from at least the first three minutes were discarded to allow stabilising of the measurement. MR_{total} was corrected for $MR_{embryos}$. Knowing the incubation stage of the embryo's of a clutch, $MR_{embryos}$ could be derived using data from Vleck *et al.* (1980). In figure 1c in their study, they summarised the relative $MR_{embryos}$ in relation to the relative incubation stage for altricial birds. Knowing the maximum $MR_{embryos}$ of great tits (J.A.L. Mertens in Vleck *et al.* 1980, $MR_{embryos}$ could be derived for each egg at a particular incubation stage. Eggs from abandoned nests were assumed to be dead or incubated shortly: their metabolic rate was neglected. MR_{inc} was calculated by subtracted the average $MR_{embryos}$ of a clutch from the MR_{total} measured in the field; $MR_{inc} = MR_{total} - MR_{embryos}$. $MR_{embryos}$ 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, average values of MR_{inc} were used in the analysis.

Statistical analysis

In the dataset of oxygen consumption in 2001, two missing values occurred; one in the enlarged and one in the reduced treatment category for two different individuals. In the year 2004, data were missing on body mass (2) and on nest thickness (1). Almost all birds steadily incubated during the measurements in both years, as observed by the nest temperatures measured by temperature probes between the eggs (see above). In 2004, however, one individual was apparently standing above the eggs at intervals during one of the nights of measurement, as assessed from temperature measurements between the eggs. Excluding this individual in the model did not change the results.

All analysis 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 the two-way interaction were tested by backward elimination from the model. Three different analyses were performed, because of slightly different experimental procedures in the two years:

1) In the first analysis data were used from the first run of the experiment in 2001 to test whether clutch size manipulation (reduced, control, enlarged) affected MR_{inc}. Original clutch size, date, ambient temperature and oxygen analyser were included in the model as extra explanatory variables.

2) In the second analysis data were used from the second run of the experiment in 2004 to test whether clutch size manipulation (control and enlarged) affected MR_{inc}. Besides the previous explanatory variables, body mass during late incu-

bation and nest thickness were included in the model.

3) In the third analysis, data from both runs of the experiment were used. The main aim of this analysis was to test whether the effect of clutch size manipulation and temperature were equal for the both runs of the experiment.

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

RESULTS

First run of the experiment; second 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) was 0.55 ± 0.07 J s^{-1} at mean ambient temperatures during the night of 14.5 ± 2.4 °C. Both the experimental treatment and mean ambient temperature explained part of the variation in MR_{inc} (Table 6.2A). Post-hoc analysis revealed that females had higher MR_{inc} when incubating experimentally enlarged clutches as compared to control clutches, but had no lower MR_{inc} when incubating reduced clutches (Fig. 6.2A). MR_{inc} increased significantly as the night temperature (T_a, °C) decreased (Fig. 6.3). Other covariates such as original clutch size, date and oxygen analyser did not explain part of the variation in MR_{inc}.
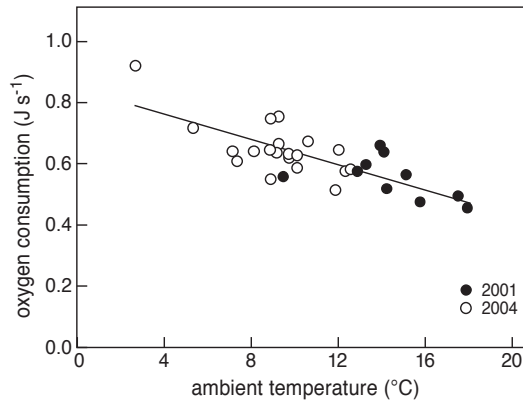
Second run of the experiment; first clutches in 2004

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 mean
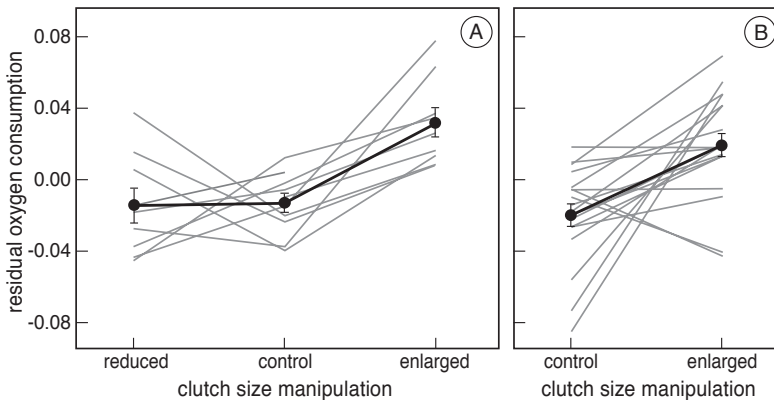
Figure 6.2. Residual MR_{inc} (J s^{-1}) - corrected for ambient temperature- of females incubating reduced, control or enlarged clutches in the year 2001 (A) and control and enlarged clutches in the year 2004 (B). The black dots represent mean value (± SE), while the grey lines represent repeated measurements of the same individual.

Table 6.2. The results of three hierarchical linear regression models of MR_{inc} ($J\ s^{-1}$) in relation to experimental treatment and several covariates. Asterisks (*) indicate whether the covariate was tested in the model and rejected, while minus signs (-) indicate that that covariate was not tested in the particular model.

	A: run 1 – 2001				B: run 2 – 2004				C: combined results			
	$\beta \pm SE$	χ^2	df	p	$\beta \pm SE$	χ^2	df	p	$\beta \pm SE$	χ^2	df	P
Intercept	0.903±0.065				0.952±0.049				0.959±0.050			
T ambient	-0.027±0.004	15.9	1	<0.001	-0.028±0.004	18.0	1	<0.001	-0.028±0.003	34.2	1	<0.001
Manipulation		14.1	2	<0.001								
- enlarged	0.054±0.015	13.5	1	<0.001	0.039±0.010	11.1	1	<0.001	0.044± 0.007	22.5	1	<0.001
- reduced	-0.011±0.015	0.6	1	NS	-	-			-	-	-	
Year	-				-0.004±0.002	3.7	1	0.05	-0.056±0.028	3.9	1	<0.001
Nest thickness	-				*				-	-		
Natural clutch size	*				*				*			
Oxygen analyser	*				*				*			
Date	*				*				*			
Body mass	-				*				-			

Figure 6.3. The metabolic rate of nocturnal incubation (MR_{inc}) for females incubating control clutches in relation to ambient temperature for two years. The line shows the regression line for both years combined.

ambient temperatures of 9.3 ± 2.4 °C during the night. Females incubated in nests with nest cups of on average 13 ± 6.9 mm thick (N_{cup}; range 3 – 29 mm) and nest rims of 56 ± 12.7 mm high (N_{rim}; range 29 – 75 mm). Height of the nest rim and thickness of the nest cup were strongly correlated: N_{rim} (mm) = 38.26 ± 4.36 (SE) + 1.37 ± 0.30 (SE)·N_{cup} (mm); R^2 = 0.56, p < 0.00, n = 19). Analyses of MR_{inc} were performed with N_{cup} as covariate. Experimental treatment, mean ambient temperature and nest thickness, all explained part of the variation in MR_{inc} (Table 6.2B). Females had higher MR_{inc} when incubating experimentally enlarged clutches as compared to control clutches (Fig. 6.2B); the effect of clutch size manipulation on MR_{inc} was similar as in the first run of the experiment. MR_{inc} increased significantly as night temperature decreased (Fig. 6.3), and thickness of the nest cup decreased. Variation in body mass did, however, not explain variation in MR_{inc}.

Combined results

MR_{inc} of females that incubated control clutches was negatively related to the ambient temperature (MR_{inc} = 0.841 (0.039) - 0.020 (0.003)· T_a (χ^2 = 24.7, df = 1, p < 0.001, n = 30; Fig. 6.3). The experimental treatment, mean ambient temperature and year explained a significant part of the variation in MR_{inc} (Table 6.2C). The effect of clutch enlargement (treatment * year; χ^2 = 0.29, *df* = 1, p = 0.59; n = 29) and the effect of ambient temperature (temperature * temperature; χ^2 = 0.09, *df* = 1, p = 0.79; n = 29) were not different for the two runs of the experiment, as indicated by the non-significance of the interaction terms. MR_{inc} of females incubating second clutches in the year 2001 was higher than that of females incubating first clutches in 2004, when controlled for ambient temperature.

DISCUSSION

Clutch size manipulation

Females spent on average 0.65 ± 0.09 J s^{-1} during nocturnal incubation (MR$_{inc}$,) during first clutches in 2004 and 0.55 ± 0.07 J s^{-1} during second clutches in 2001, at mean ambient temperatures of 9.3 ± 2.4 °C, and 14.5 ± 2.4 °C, respectively. Females spent energy at a higher rate when incubating enlarged clutches as compared to control clutches, but they did not spend energy at a lower rate when incubating reduced clutches. The effect of clutch enlargement relative to the mean (MR$_{inc}$) in each of the two years (6- 10%; 3 additional eggs) was similar in both years. Ambient temperature explained the largest part of the variation in MR$_{inc}$ (43% - 49% per 10 °C; mean $\pm$ SD; 11.1 ± 3.4). Nest thickness (6% per 10 mm; 13.1 ± 6.8) and year (or breeding attempt; 9% relative to the mean MR$_{inc}$ of the two years combined) explained an additional part of the variation in MR$_{inc}$.

   Clutch size manipulation can affect the metabolic rate of contact incubation by three possible mechanisms. Firstly, keeping any extra egg warm may take more energy, because the surface over which heat can be lost might increase with clutch size. Secondly, clutch enlargement likely affects the size of the nest cup. At a certain point, the incubating female may not be able to maintain her upward insulation resulting in heat loss through convection, and consequently, peripheral eggs may cool. And thirdly, above a certain clutch size ('threshold clutch size') some eggs may have incomplete contact with the female's brood patch (Mertens 1977) and cool during incubation of the other eggs. In both latter cases, the incubating bird needs to repeatedly rearrange the eggs and rewarm the cooled eggs. Rewarming of eggs is more costly than maintaining eggs at incubation temperatures (Biebach 1986; Vleck 1981). Given the fact that the mean clutch size in the year 2001 was similar to the threshold clutch size for great tits (Mertens 1977), these three mechanisms, and especially the second and third mechanism, may explain why clutch enlargement had a larger effect on MR$_{inc}$ than clutch reduction.

   All studies that measured the clutch size related metabolic rate during nocturnal incubation so far (Biebach 1981; Biebach 1984; Haftorn and Reinertsen 1985; Weathers 1985) found that clutch size manipulation affects MR$_{inc}$, independent of whether measurements were performed under laboratory or field conditions (Table 6.3). Moreover, the studies found that clutch size explained a similar proportion of variation in MR$_{inc}$ when expressed as one-third of the mean clutch size for that species, and assuming linearity (Fig. 6.4). Of the previous studies, Biebach (1981; 1984) measured the effect of clutch size for a whole range of clutch sizes, and thus measured the shape of the relationship between MR$_{inc}$ and clutch size. In contrast to our finding, he found a linear relationship between MR$_{inc}$ and clutch size. A possible explanation for this difference in the shape of the relationship between MR$_{inc}$ and clutch size is that the mechanism with which clutch size affects MR$_{inc}$ differs between species. For instance, the surface over which heat is

Table 6.3. Overview of studies that determine clutch size related energy expenditure of nocturnal incubation (MR_{inc}) in passerines .

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 *Serinus canaria*	20.8	reduced [b]	-2/-3	3	3-15	1-5	Weathers 1985
Laboratory	european starling *Sturnus vulgaris*	80	continue [a]	1-8	?	3-5	3-5	Biebach 1981
Laboratory	european starling *Sturnus vulgaris*	80	continue [a]	1-8	8	3-5	3-5	Biebach 1984
Field	blue tit *Parus caeruleus*	11.5	reduced [b]	-5	1	18	4	Haftorn & Reinersten 1985
Field	great tit *Parus major*	20.2	reduced [b]	-2/-3	9, 0 [c]	0 (n.s)	0	This study
			enlarged [b]	3	9, 20 [c]	6-10	2-3	

[a] clutch sizes was manipulated in steps of one egg over a range from 1 to 8 eggs
[b] clutch size manipulation relative to control
[c] sample size given separately for two years of measurement
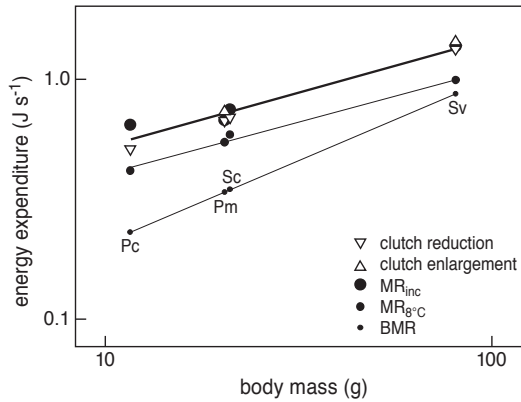
Figure 6.4. An overview of 5 studies on 4 passerine species (*Parus caeruleus* [Pc], *Parus major* [Pm], *Serinus canaria* [Sc], and *Sturnus vulgaris* [Sv]) that determined the effect of either clutch reduction and/or clutch enlargement on metabolic rate during nocturnal incubation (MR_{inc}, $J\ s^{-1}$) in comparison to MR_{inc} when females incubated control clutches (see also Table 6.3). For comparison, the effect of clutch size on MR_{inc} is expressed as one-third of the mean clutch size for that specific species. MR_{inc} at ambient temperature of 8°C is shown as a function of body mass (gram). Both the relationship between the metabolic rate at rest at 8 °C of non-incubating birds ($MR_{8°C}$) and body mass, and basal metabolic rate (BMR) and body mass are given for reference.

lost may be larger in starlings *Sturnus vulgaris*, because not only starling eggs are larger than great tit eggs, but also nests of starlings may be of inferior insulation as compared to those of great tits (Reid *et al.* 1999). In this study, birds with thicker nests, and thus better insulated nests (Szentirmai *et al.* 2005; chapter 5), had lower MR_{inc}.

Because we did perform the experiment under field conditions, we can compare the effect of the experimental treatment to the effects of other factors influencing MR_{inc}. The effect of clutch size on MR_{inc} is relatively small in comparison to that of ambient temperature. Ambient temperature influenced MR_{inc} strongly; with an increase in ambient temperature of 10 °C, MR_{inc} decreased by 43-49%. Such an increase in ambient temperature is likely to occur in the course of the incubation phase (Fig. 6.3). In both runs of the experiment, thus when females incubated either first clutches early in spring (temperature range 2.8 – 12.6 °C) or second clutches later in spring (temperature range 9.6 – 18.0 °C), they experienced ambient temperature that fluctuated in the order of magnitude of 10 °C. Moreover, the lower critical temperature - the temperature below which individuals have to spend energy on homoiothermy- is higher for incubating birds (Haftorn and Reinertsen 1985; Williams 1996). Using the equation given for females incubating control clutches (MR_{inc} [$J\ s^{-1}$] = 0.844 - 0.021·T_a [°C]) in combination with the calculated BMR (0.34 $J\ s^{-1}$), the lower critical temperature was estimated to be 24.3 °C. This means that even birds with late clutches experi-

enced ambient temperatures below thermoneutrality during nocturnal incubation. These results show that ambient temperature is an important source of variation in the energetic costs of incubation.

Incubation versus non-incubation: comparison between species

Whether the level of energy expenditure during the incubation of eggs exceeds that of resting metabolism has long been subject of debate (Kendeigh 1963; King 1973; Mertens 1977; Walsberg and King 1978). To answer this question, the best option would be to have direct measurements of the metabolic rate during incubation (MR_{inc}, J s^{-1}) and basal metabolic rate (BMR, J s^{-1}) for the same individuals. Measuring the BMR for incubating female great tits is, however, cumbersome as they are prone to desert the nest after handling in the incubation phase. Therefore, data from literature was used to approximate BMR for incubating females in this study. BMR was calculated for a female of on average 20.2 gram, using Kendeigh *et al.* (1977; see Methods). Subsequently, MR_{inc} – standardising for ambient temperature of 8 °C (c.f. Tinbergen and Williams 2002) – was calculated as a multiple of BMR to facilitate comparison between species. The value of 2.0 times BMR found for this study falls within the range of values (1.6 to 3.0) that have previously been reported for a number of passerines (Fig. 6.4; for an overview see Tinbergen and Williams 2002).

Incubation generally takes place at temperatures below thermoneutrality and, therefore, expressing MR_{inc} as a multiple of metabolic rate at rest but at the same temperature as during incubation (MR, J s^{-1}) instead of BMR would be a more useful comparison. Data on the relation between MR and ambient temperature exist for non-incubating great tits during the breeding season (Mertens 1980) and during winter (Broggi *et al.* 2004). MR_{inc} exceeds MR by 1.2 to 1.6 times, respectively (Fig. 6.4), when both measures were standardised at 8 °C (here abbreviated as $MR_{8°C}$). Examination of the curve for MR_{inc} versus body mass (Fig. 6.4) reveals that smaller bird species have relatively high MR_{inc} in comparison to that of larger ones. The high MR_{inc} can be explained by the investment needed for thermoregulation; the curve of $MR_{8°C}$ in relation to body mass shows higher $MR_{8°C}$ for smaller bird species (Broggi *et al.* 2004; see also Williams and Tieleman 2000).

Implications

During nocturnal incubation, females spent energy at a higher rate when incubating enlarged clutches as compared to both control and reduced clutches. Due to these clutch size related energetic costs during nocturnal incubation, daily energy expenditure is expected to increase with clutch enlargement. Furthermore, ambient temperature explained most of the variation in MR_{inc}. For small bird species energy expenditure during incubation is relatively high, which is mainly due to high energetic costs of thermoregulation. These results imply that the incubation phase may be energetically costly due to its timing in the season, as a result of its

position in the sequence of reproduction (egg laying, incubation and nestling phase). Especially in temperate zones with seasonality, the energetic costs of reproduction must be high given that ambient temperatures are low and unpredictable early in the season (Perrins 1970). Ambient temperature may, therefore, be an important factor constraining early reproduction (Stevenson and Bryant 2000), especially in small bird species.