ENERGETIC COST OF INCUBATION

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SUMMARY

We measured energy expenditure in free living incubating Great tits (*Parus major*) by doing oxygen measurements during the night. We measured the same birds two nights in a row and one of the nights we manipulated the clutch size of the bird. Evidence was found that oxygen consumption was dependent on temperature, manipulation and nest thickness.

INTRODUCTION

A lot of research exists on reproduction because reproduction is an energetically expensive period in the annual cycle of a bird. We expect that energy spent in this period trades off with the future reproductive output of birds (Tinbergen & Williams, 2002). During evolution clutch size is determined by selection, and we expect that the number of chicks produced reflects the maximum number that the parents can provide food for (Lack, 1948). Or more precisely we expect the family size to maximize fitness. During the reproductive phase there are three important stages for a bird, egg laying, incubation and chick rearing.
All attention in most of the studies done so far was directed to the chick-rearing phase because this was expected to be the most expensive phase (both in terms of energy and in terms of fitness) for a bird. (Lack, 1948; Cichoń, 2000;Visser and Lessells, 2001; Hõrak, 2003; Thomson *et al.,* 1998 e.g.). The reason that incubation was not expected to be as expensive as the chick-rearing phase is because the heat needed to maintain egg temperature could perhaps be supplied from a bird's obligatory basal heat production (King, 1973). In an overview of different studies on breeding birds, 23 studies on 19 species found no differences between the incubating metabolic rate (IMR) and the resting metabolic rate (RMR) of a bird. But on the other hand 15 studies on 15 species found that IMR was greater than RMR (Thomson, 1998 e.g.).
A pioneer in thinking about energy expenditure during incubation, Kendeigh (1963), developed a model that female house wrens expended 1.2 tot 2.1 times their basal metabolism while sitting on eggs. Biebach (1981, 1984) measured the oxygen consumption of incubating starlings, incubating in nest-boxes in a laboratory, while manipulating clutch size and ambient temperature. He found that the energy expenditure of the starling increased with larger clutch sizes (also Coleman and Whittall, 1988; Moreno and Sanz, 1994; Weathers, 1985; Moreno *et al.*, 1991) and lower ambient temperatures. Haftorn and Reinertsen (1985) also found that a decreased ambient temperature raised the energy expenditure in incubating Blue tits. They measured this by manipulating the temperatures of the nest boxes and found that the oxygen use is higher when the female is incubating and lower when she stands above the eggs. By reducing the clutch size of an incubating Blue tit by 5 eggs they found that oxygen consumption decreases by eighteen percent. Another aspect found is that the lower critical temperature of the zone of termo neutrality shifts toward higher temperatures with increasing clutch size (Mertens, 1977). For a Great tit incubating on seven or more eggs the lower critical temperature is 24 degrees Celsius.
The current work is part of a larger project that investigates how important the incubation phase is for the parental birds in making decisions on clutch size. The specific question is: What is the energetic cost of incubation? We measured the energetic cost of incubation by doing oxygen measurements on incubating Great tits at night. Oxygen measurements were done in the field by measuring the same bird two nights in a row where we manipulated clutch size randomly during the first or the second night. The ambient temperature was not manipulated. We found that a decrease in ambient temperature and an enlargement of the clutch size entailed higher oxygen consumption of an incubating Great tit.

METHODS

Study area and study species

The study was conducted in a Great tit population in the Lauwersmeer in the Netherlands (53°20' N, 06°12' E). There were eight forests where in total 200 nest- boxes were placed in different densities. The forests were relatively young and that's why there were almost no natural holes where the hole breeding Great tit could breed. The great tit is a small eighteen-gram passerine bird and has an average clutch size of 9 to 10 eggs. In the Lauwersmeer the Great tit sometimes has a second clutch. Only the female incubates the eggs and sometimes the male helps the female by bringing food to the nest. There is already evidence that the parents can raise more young then they actually do (Tinbergen and Sanz, 2004) making it interesting to see whether the incubation phase may be responsible for an extra fitness cost preventing the tits to lay more eggs.

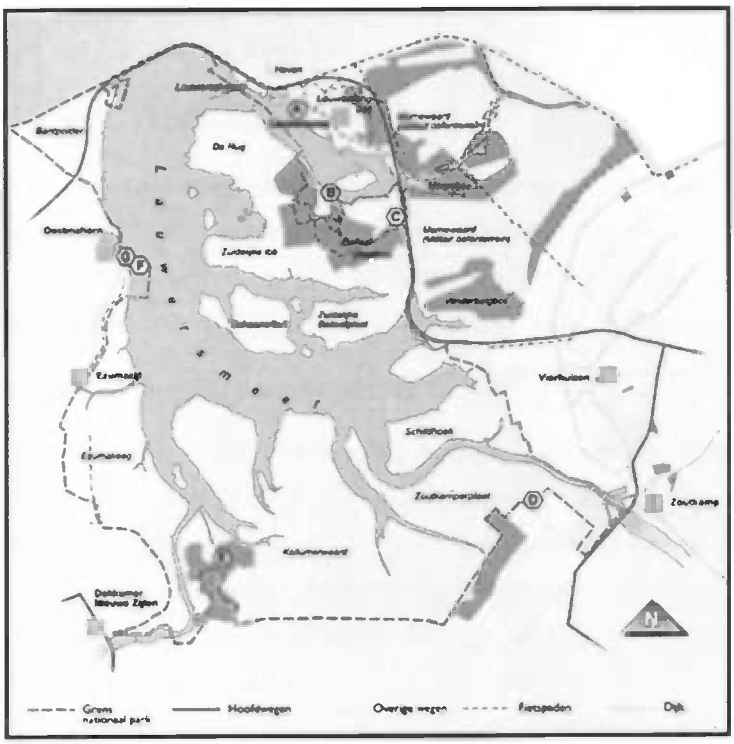

Figure 1: An overview of the study area.

General procedures

In the breeding season of 2004 all boxes were checked once a week from the first of April till the end of June. In this way it was possible to determine the onset of egg laying. The Great tit, when laying, generally lays an egg early in the morning every day. By counting back the number of eggs it is possible to determine the onset of egg laying assuming that no birds skipped days. To determine the onset of incubation nests were visited every day from the day the 6^{th} egg was expected.

To determine the hatching date the nest-boxes were checked daily, starting two days before hatching was expected (first day of incubation + 12 days). Between day 7 and 11 of the chick rearing phase (day 0 = day of hatching) the parents were caught to identify them and measure tarsus, wing length, weight. They were color banded or ringed when necessary. At the same occasion the chicks were ringed and weighed. On day 14 of the chick-rearing phase the wing length, tarsus and weight of the chicks were taken. To check whether the chicks had fledged, the nest-box was checked every two days starting from expected fledging date. The nest was removed from the nest-box when the chicks were fledged and there were no new eggs in the box.

Energetic Cost of Incubation

We measured the oxygen consumption of the same individual two nights in a row with the same equipment between the 2^{nd} and 6^{th} night after incubation started. Before the first or second night we randomly manipulated the clutch size though adding three eggs. By doing the experiment in this way each bird was its own control. The ambient temperature was not manipulated during the experiment.

Preparation Oxygen measurements

Two (or one) days before measuring the oxygen consumption the nest-box was modified into a small metabolic chamber. To make sure that the nest-box was airtight a mouse pad was placed between the lid of the box and the box itself. At the start of the measurements a cork was placed in the entrance hole. A few little holes were made in the bottom and one at the side (with a tube) of the nest-box so that respectively air could get in from the outside at the bottom and could be pulled out at the side by the oxygen analyzer during oxygen measurements.

Manipulation experiment

Every bird was measured two times. One of the nights the Great tit had to incubate three additional eggs, enlarged, and the other night the female great tit had to incubate her original clutch size, control. By doing the experiment in this way the bird was her own control. These manipulations were done (in random order) before the first or second night of measuring. No reduced clutch size measurement was used because earlier manipulation studies in this population revealed that females with reduced clutches were more likely to abandoned their nest (M.E. de Heij, pers. comm.).
The additional eggs were taken from a donor nest with eggs of about the same age and marked for recognition. This donor nest received three fake eggs in return to keep the number of eggs constant. After the measurements the marked eggs were put back in the nest where they were taken from and the fake eggs were removed from the donor nest.

Oxygen Measurement

The energetic cost of incubation was determined by doing oxygen measurements with a two-channel, car battery powered, transportable respirometer. This was taken out during the night because the Great tit does not leave the nest-box after 9 PM and before 5 AM (M.E. de Heij, pers. comm.). The oxygen measurement equipment (machine 1 & 2, servomex model 570A, Crowborough, UK) was installed between ten and eleven PM about two to three meters away from the nest-box. After an hour of warming up, to create stable air in the drier tubes, the machines were calibrated with dry nitrogen to create a zero point. Afterwards, the so-called span was calibrated with outside air. These calibrations were also done after the measurements. Machine 1 measured for 60 minutes oxygen consumption of the Great tit and for 10 minutes outside air. Machine 2 did this respectively for 24/24 minutes. The airflow of both machines through the drier tubes was around 20 liters an hour. Both the machines measured for at least 2 hours in total the oxygen consumption of the incubating female Great tit. The machines also had temperature probes measuring the ambient temperature, the temperature in the nest-box and the temperature of the oxygen analyzer. In total we did 40 oxygen measurement on 20 different individuals. We recorded: Oxygen consumption (%), airflow (% and mV), ambient temperature, temperature inside the nest-box, temperature of the machines with a logger (machine 1: Campbell Scientific Inc. CR10X and machine 2: Squirrel 1202). The energetic cost of incubation was calculated from the oxygen consumption. We used an RQ of 0.706 and for the Energy equivalent for O_2 consumption we used 19.7 kJ/L (Hill, 1972).

Nest measurements

Nest thickness was measured once during incubation in 2004. A knitting needle was used as measuring tool that pierced the nest material. The distance between top of the nest material and the bottom of the nest was taken as measure of nest depth. This distance was measured to the nearest millimeter with a small caliper. We measured at five different positions in the nest, one in each corner and one in the center of the nest to determine nest shape and thickness.

Calibrations

Temperature calibration

After the measurements in the field all temperature probes were calibrated in the lab. All probes and a calibration probe (digital temperature probe) were placed in a homogeneous water solution of almost 40 degrees Celsius. Subsequently the water was cooled by ice till 5 degrees Celsius. Every minute the temperature of all probes was recorded. Every probe's measurement was corrected on the basis of these calibrations. For detailed information how the temperature probes differ from the calibration probe see figures 6 and 7 in the appendix.

Oxygen-consumption calibration

At the end of the experiment we calibrated the field oxygen measurement equipment (machine 1 and 2) for oxygen consumption by calibrating it with the oxygen measurement equipment at the lab (machine 3) which is precise at 0.003% (Overkamp pers. comm). During the calibration the three machines measured the oxygen consumption of the same animal by pulling air out of the same metabolic chamber at the same time. Machines 1 & 2 were installed in a temperature-regulated room and machine 3 was kept at a constant temperature of 21 degrees Celsius. Machines 1&2 were calibrated at three temperatures, respectively 7, 13 and 20 degrees Celsius, for 2 times. We did this because we wanted to make a reliable calibration range. By calibrating the machines 1&2 we found that machine 1 measured a to high oxygen consumption and that machine 2 measured about the same or a little bit lower oxygen consumption. See figure 5 in appendix. The values used in this report have not been adjusted yet due to time limitation. However the differences between machine 1 & 2 are close to the differences we estimate from our field measurements (respectively 0.1% during calibration and 0.1% of the oxygen consumption in the field).

Data Analysis and Statistics

We used the General Linear Model - Univariate (SPSS 11.5) to analyze the data. We used Oxygen consumption as the dependent variable, machine as random factor, manipulation as fixed factor and temperature and nest thickness as a covariate. Other programs, which are used to calculate and register data, are Microsoft Access 97, Excel 97 and Word 97.

It is also found that eggs use oxygen as well when they are longer incubated (Haftorn and Reinertsen, 1985; Gloutney *et. al.*, 1996). That's why we measured the oxygen consumption as early as possible during the incubation period. But we also tested if the age of the incubated eggs had an influence on the oxygen consumption we measured. We found no significant results (GLM, $n = 38$, $F = 0.037$, $p = 0.971$). Due to a missing nest measurement we lost two oxygen measurements of the same individual (control and enlarged).

RESULTS

Energetic Cost of Incubation

Energy expenditure during night incubation was related to ambient temperature, clutch manipulation and nest thickness (see table 1). A very important variable explaining the energy expenditure was the ambient temperature. (GLM temperature effect controlled for manipulation and nest thickness: n = 38, F = 40.215, p = 0.000).

Table 1: Variable's which are tested (GLM-UNIVARIATE, SPSS).

Parameter	B	SE	df	F	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	.993	.051	1, 33	212.281	.000	.889	1.098
Ambient Temperature	-.029	.005	1, 33	40.215	.000	-.038	-.020
Machine	.104	.019	1, 33	30.632	.000	.066	.143
Manipulation	-.041	.019	1, 33	4.940	.033	-.079	-.004
Nest thickness	-.004	.001	1, 33	7.673	.009	-.007	-.001

The age of the eggs was included in the analysis but gave no significant results.
Machine 1 measured a 0.104 higher oxygen consumption than machine 2. Manipulation by enlarging clutch size by 3 eggs gave a 0.041 more oxygen consumption.

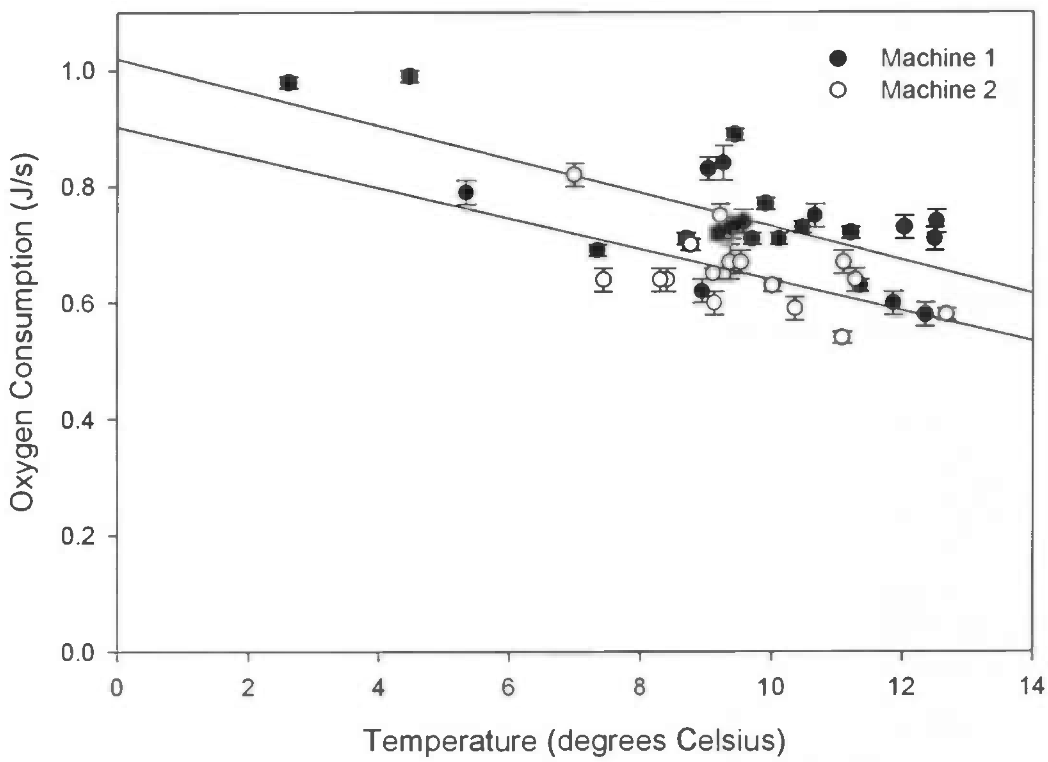

Figure 2: Uncorrected measurements of the oxygen measurements. We found that lower temperatures increased the oxygen consumption of an incubating Great tit. (GLM, n = 38, F = 40.215, p = 0.000)

Manipulation Experiment

The control measurement (n=17) revealed lower energy expenditure than the enlarged measurement (n=17). An incubating female had to spend 0.041 (+/- 0.019) J/s more when incubating a large clutch than control. (GLM: n=38, F=4.940, p=0.033 controlled for ambient temperature and machine). This means that an enlarged nest costs more energy to incubate than incubating a normal clutch size.

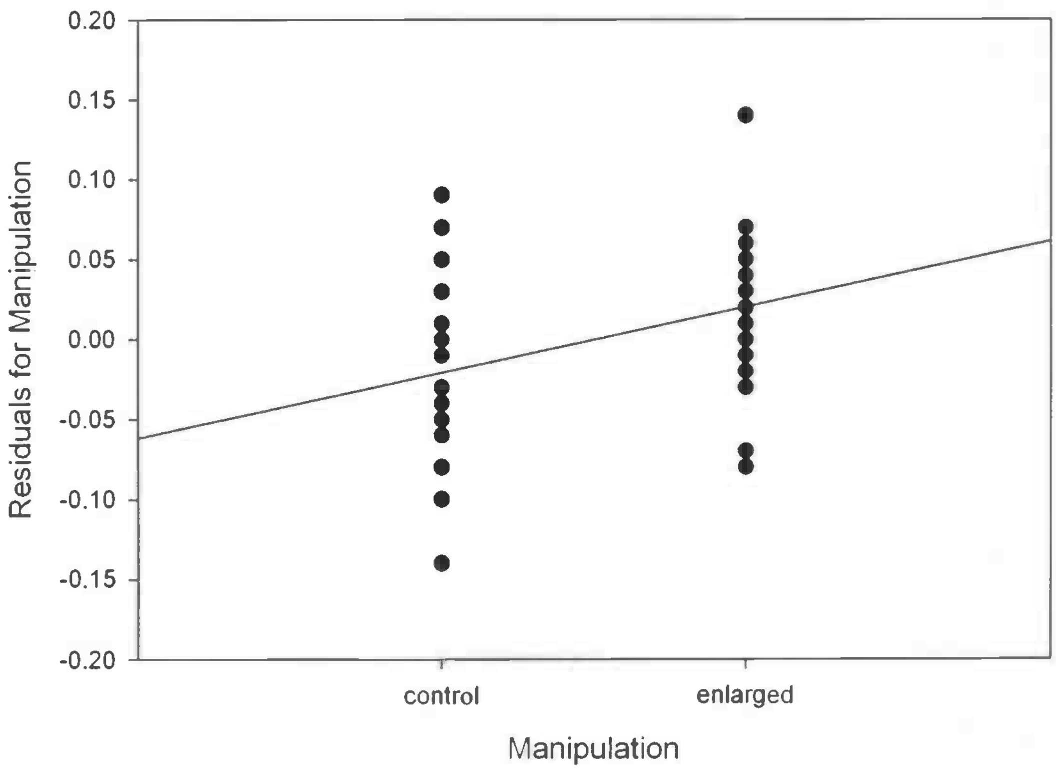

Figure 3: Manipulation against the residuals for manipulation. An enlargement of three eggs had a significant influence on the oxygen consumption. GLM: n=38, F=4.940, p=0.033 controlled for ambient temperature and machine.

Nest measurements

The thickness of the nests had a significant influence on the energy expenditure of the great tit. For the average thickness of the nest mid there was a correlation that the incubating birds with thicker nest use less oxygen (energy) to incubate the eggs. (GLM: n=19, F=7.673, p=0.009).

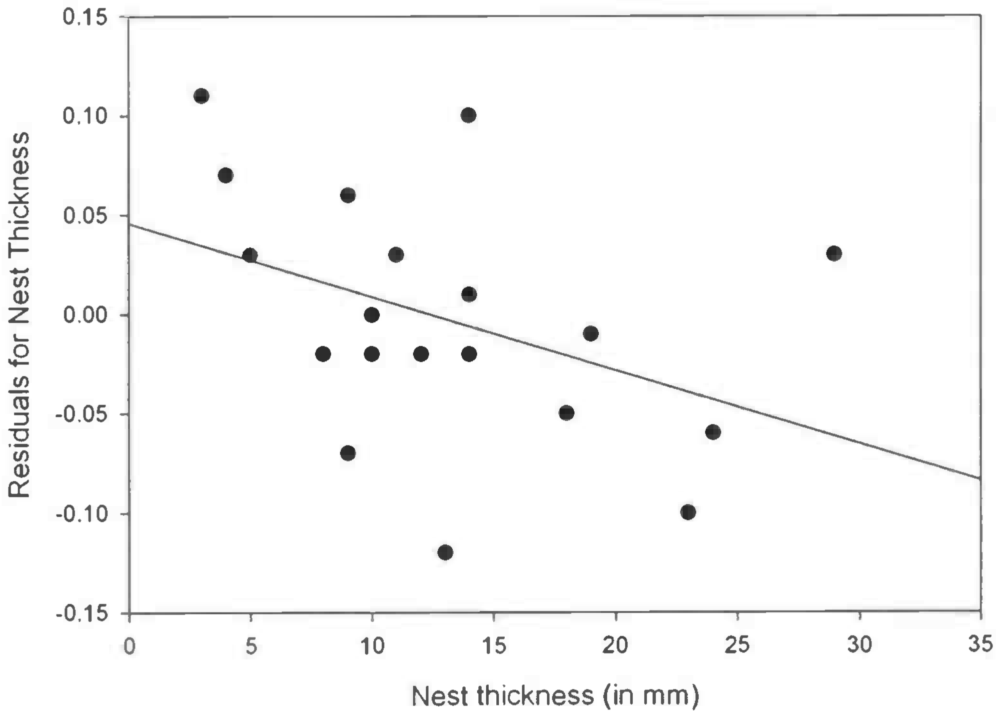

Figure 4: The residuals of nest thickness against the nest thickness. Controlled for ambient temperature and manipulation. (GLM: n=19, F=7.673, p=0.009).

DISCUSSION

In this study we found that the oxygen consumption of an incubating Great tit increased with decreasing ambient temperature. Also the Great tit incubating on larger than normal clutch sizes invested more energy in incubation. Also thicker nests had lower energy expenditure.

Energetic Cost of Incubation

We found an incubating metabolic rate (IMR) between 0.59 and 0.87 J/s (related to machine 2) for a Great tit. This means that a Great tit spent 2 till 3 times as much energy incubating a clutch then post adsorptive in rest at termo neutrality (basal metabolic rate or BMR, Mertens 1977). Our data on the oxygen consumption of incubation by a free living Great tit supports the view of Kendeigh (1963) and Haftorn (1985) that incubation, of small birds, requires the production of additional heat when the temperature is below the lower critical temperature of the termo neutrality zone.
Since the temperature in April was between 2 and 14 degrees Celsius which is below the zone of termo neutrality of the Great tit (Mertens, 1977), the energy expenditure was higher than BMR and depended on the temperature. During daytime when a Great tit was off the nest she had to re-warm the eggs when she came back to the nest. Rewarming eggs is also an expensive task for the bird (Biebach, 1986). By measuring at night we kept the cost of rewarming eggs to a minimum. Potentially the costs of rewarming the eggs may cause the energetic costs during daytime to exceed the nighttime incubation costs. This could increase the energetic costs during incubation to the level measured during the chick-rearing phase. An interesting question will be how much energy the bird spends during the day including flying, feeding, incubating and rewarming the eggs by measuring the energy expenditure with use of DLW.

Manipulation

If optimal clutch size is determined only by the costs incurred during the chick rearing phase, we would not expect higher oxygen consumption when enlarging the clutch size during incubation. But, we found that a Great tit with an enlarged clutch size had to invest more energy then she had to do on her original clutch size. The conclusion is that incubation is more costly when incubating more eggs just like Monaghan and Nager wrote in their review. The increase of clutch size can result in lower hatching success, longer incubation, decrease in body condition of the incubating bird (e.g. Monaghan and Nager, 1997) and as we found higher energy expenditure of the incubating bird.

Nest thickness

We found that the oxygen consumption was also significantly related to nest thickness. Incubating Great tits with thicker nests had to invest less energy then do Great tits with thin nests. This could mean that when the tits with thicker nests leave this, eggs would not cool down as much. And because of that the bird doesn't have to invest so much energy to re-warm the eggs. Estimates of steady state incubation costs based on the effect of nest thickness on cooling rate of a plaster ball in the nest were in the same order of magnitude as we found here. For our study this means that birds with thicker nests will not have to invest as much energy as birds with a thinner nest. The Great tit could save energy during incubation when she would build a thicker nest. But building a nest is a process that also costs energy. The Great tit has to trade off between building a thicker nest and spending more energy during incubation. It is found that female Great tit building a nest had an increase of energy expenditure of 12 % (Nilsson and Råberg, 2001).

The question was: What is the energetic cost of incubation. We found that a Great tit incubating at temperatures below the Termo Neutrality Zone (TNZ), and incubating more eggs had to invest more energy to incubate the eggs. Building a thicker nest can also help the bird in expending less energy. For the female Great tit this means that it has to trade off between building thicker nest and more costly incubation. But also between how many eggs the Great tit lays in order to keep the energetic cost of incubation to a tolerable level in addition to how many chicks can be raised.

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APPENDIX

Calibration of Oxygen Consumption

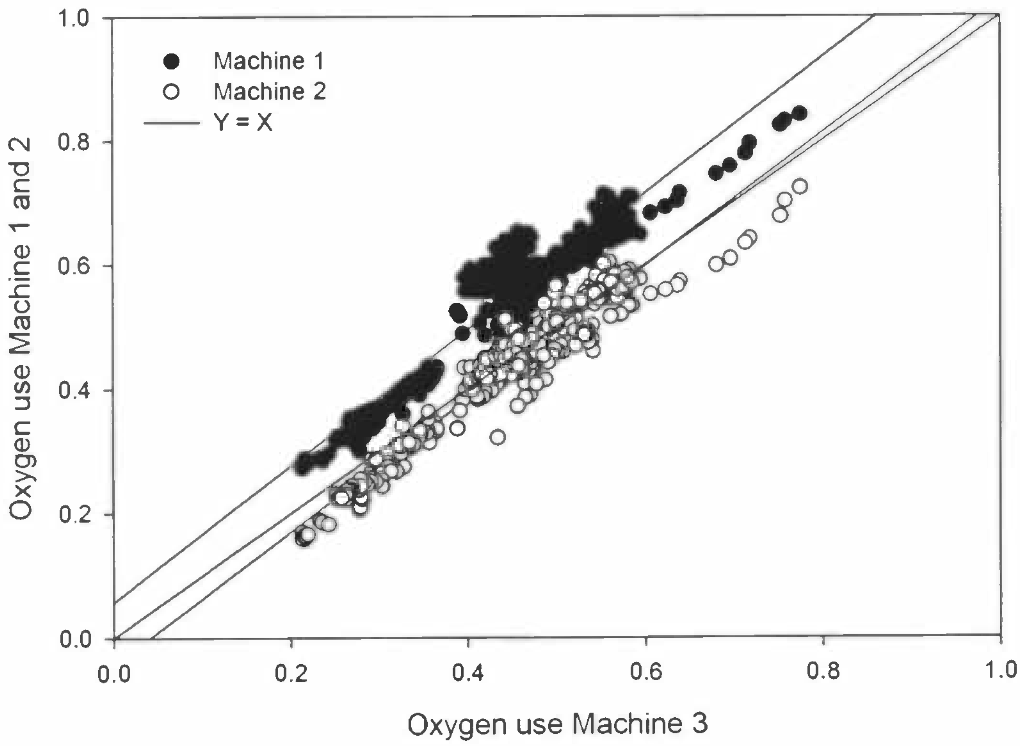

Figure 5: The oxygen consumption of field oxygenmeasurement equipment (machine 1 and 2) plotted against the laboratory oxygen measurement equipment (machine 3) which has an accuracy of 0.003 % (Overkamp, pers. comm.). Machine 1 measured a to high oxygen consumption but due to time limitations the values of the oxygen consumption are not corrected yet.

Calibration of Temperature

All the temperature probes of both machines were calibrated with a calibrated temperature probe at the same time. This is done during two calibration sessions (1 and 2).

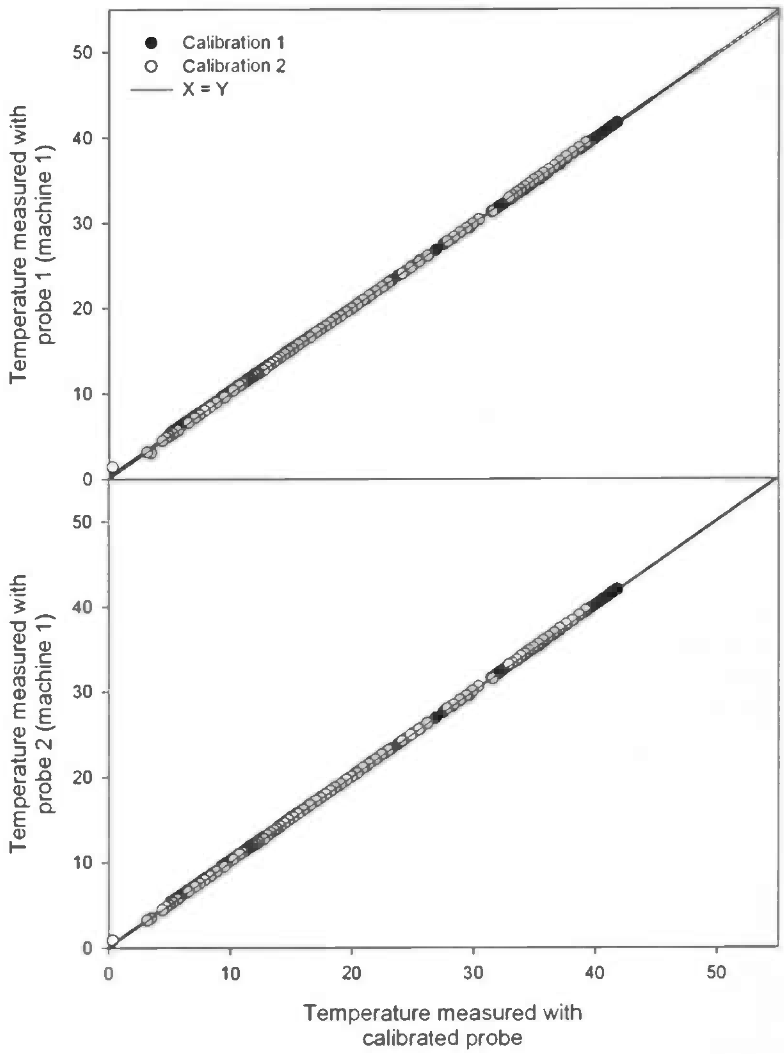

Figure 6: Calibration of temperature probes machine 1 against the real temperature. The measurements of both probes nearly measure the same as the calibrated probe.

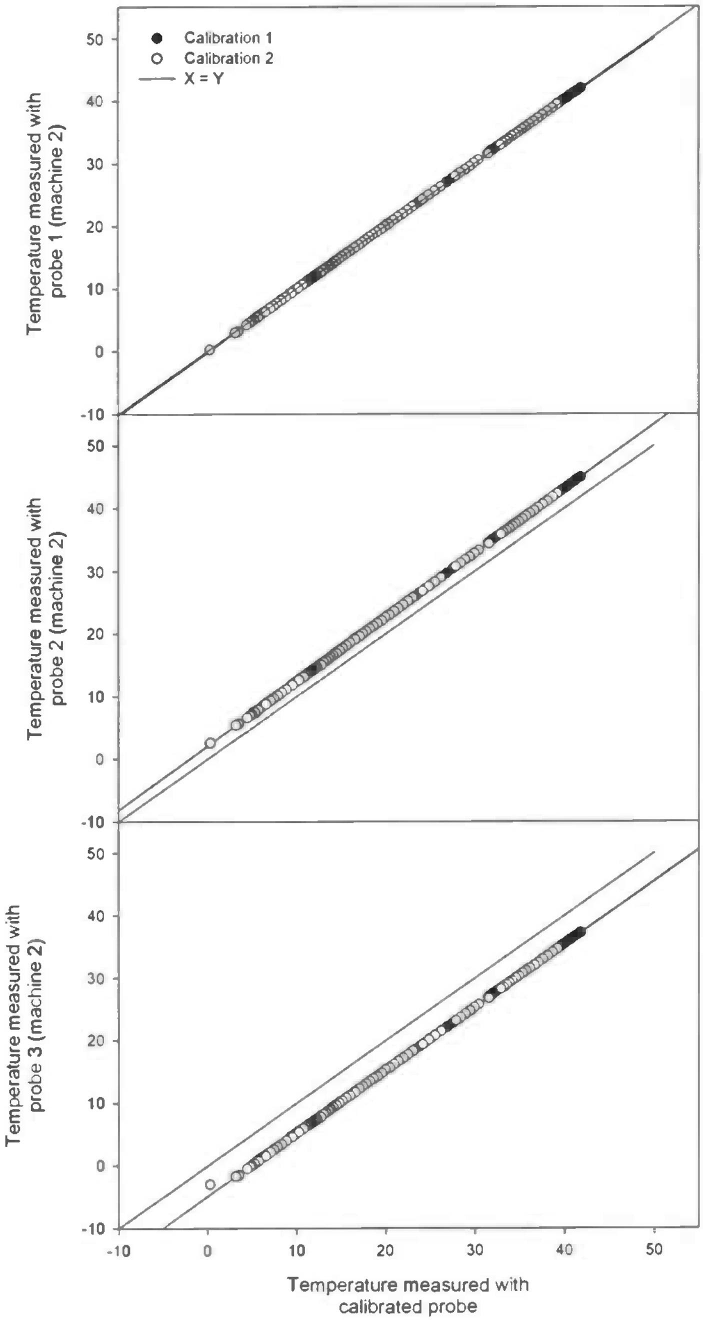

Figure 7: Calibration of temperature probes machine 2 against the real temperature. The measurements of both probes nearly measure the same as the calibrated probe.

As can be seen in the graphs before not al temperature probes measured the correct temperature. That was a reason for us to correct the temperatures we measured in the field. All the temperatures used in this article were corrected to real temperatures.