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Costs of avian incubation

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Offspring performance is negatively affected by clutch enlargement during incubation

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Unpublished manuscript

ABSTRACT

Recent studies on clutch size decisions in avian life history reveal fitness effects during incubation especially for enlarged clutches. These fitness costs may arise in two ways: *via the parents* or *via the eggs*. In this paper, I discuss how effects via parents and via eggs can be separated by using a particular scheme to exchanging eggs between nests; eggs of one nests ended up in all three treatment categories during incubation, while at the same time parents either incubated reduced, control or enlarged clutches. Next, I present the data of clutch size manipulations performed in great tits (*Parus major*) during two breeding seasons on both pre-hatching performance and post-hatching performance of offspring using this manipulation scheme. Using of a within-clutch comparison, I found that in one of the two years eggs incubated in experimentally enlarged clutches had longer developmental time than eggs incubated in reduced and control clutches. Yet, in both years nestlings that hatched from eggs incubated in enlarged clutches had shorter tarsi than those hatched from eggs incubated in either control or reduced clutches. When structural size of an individual is related to fitness, these findings indicate that eggs incubated in enlarged clutches suffer developmental detriments. Thereby, this study is the first to show negative effects via eggs of clutch enlargement during incubation on post-hatching performance of offspring. This finding should be bared in mind when performing clutch size manipulations in future.

INTRODUCTION

Natural selection on clutch size in birds may act during the egg laying, incubating or nestling phase. The general approach to isolate fitness consequences of a reproductive phase has been to manipulate the parental investment during one or more phases (Fig. 3.1) and to measure the consequences. The fitness consequences of such manipulations have been measured on parents and their offspring, because in iteroparous species parents need to trade-off investment in the current breeding attempt with self-maintenance (Williams 1966; Charnov and Krebs 1974).

Most attention has been paid to the nestling phase (Lindén and Møller 1989; Dijkstra *et al.* 1990; Lessells 1991; Vanderwerf 1992); rearing offspring has traditionally been assumed to be energetically more costly for parents than laying or incubating eggs. An increasing number of studies, however, shows that energy expenditure of females incubating eggs is similar to that of females rearing offspring (Williams 1996; Tinbergen and Williams 2002) and thus that the costs of incubation may be important for clutch size decisions. This finding has stimulated experiments that manipulate the number of eggs during incubation.

Two types of such experiments can be distinguished that differ in whether eggs are returned at hatching or not (summarised in Fig. 3.1 and Table 3.1). In the first type, clutches receive extra eggs at the beginning of the incubation period and these eggs are not returned to their original clutches at hatching (Fig. 3.1, type B). Under this type of manipulation experiments, two subtypes can be identified on the basis of the type of control used (Fig. 3.1, subtype B₁ and B₂). With subtype B₁, the true costs of incubation can only be studied till hatching. After hatching, the costs of incubation cannot be separated from that of rearing nestlings (Sanz 1997; Hanssen *et al.* 2003; Hanssen *et al.* 2005; Table 3.1), because the performance of parents and offspring of manipulated clutches is compared with that of clutches that were unmanipulated during the incubation phase. In the other subtype (B₂), the performance of parents and offspring of manipulated clutches during incubation is compared with that of clutches that receive additional nestlings in the nestling phase. When this method is used, effects can be ascribed directly to the costs of incubation (Heaney and Monaghan 1995; Visser and Lessells 2001; Table 3.1).

In the second type of manipulation experiments (type C), clutches receive extra eggs at the beginning of the incubation period and these eggs are returned to their original clutch at hatching; either just prior to (Heaney and Monaghan 1996; Reid *et al.* 2000b; Cichon 2000; Engstrand and Bryant 2002) or directly after hatching (Ilmonen *et al.* 2002). In this case, the performance of parents and offspring of manipulated clutches is compared with that of clutches that kept their original clutch size during incubation (Fig. 3.1, type C).

Experiments have revealed fitness consequences of clutch enlargement for both parents and offspring. Females incubating enlarged clutches have increased ener-

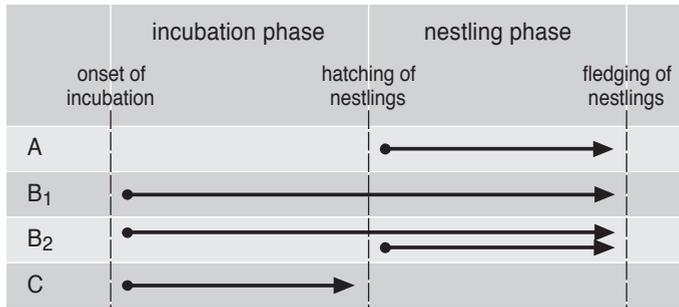


Figure 3.1. Types of manipulation experiments; with manipulation type A the costs of nestling rearing can be isolated; with manipulation type B the costs of incubating can be studied till hatching. The costs of incubation for parents and offspring after hatching cannot (B₁) or can be (B₂) isolated from that of rearing nestlings, depending on the control category; with manipulation type C the costs of incubation are isolated.

gy expenditure (Biebach 1981; Biebach 1984; Haftorn and Reinertsen 1985; Weathers 1985; chapter 6), reduced immune function (Hanssen *et al.* 2005) or reduced condition (Moreno and Carlson 1989) during incubation. Females incubating enlarged clutches had lower probability to survival locally in the subsequent breeding season (Visser and Lessells 2001; chapter 2). Eggs incubated in enlarged clutches have prolonged incubation period (Moreno and Carlson 1989; Smith 1989; Szekely *et al.* 1994; Siikamäki 1995; Engstrand and Bryant 2002), increased hatching asynchrony (Moreno and Carlson 1989) or reduced hatching probability (Moreno *et al.* 1991; Siikamäki 1995; Reid *et al.* 2000b; Engstrand and Bryant 2002). Post-hatching performance of offspring was also negatively affected by clutch enlargement. Offspring have reduced body condition (Reid *et al.* 2000b; Cichon 2000) and reduced fledging probability (Heaney and Monaghan 1995; Sanz 1997; Ilmonen *et al.* 2002). The recruitment probability of these offspring was not affected by clutch enlargement during incubation (Visser and Lessells 2001; chapter 2). It is too early to tell whether the results of the two types of experiments (B – especially subtype B₂ – and C) differ from each other.

Effects of clutch size manipulations on the pre-hatching performance (i.e. developmental time and hatching probability) of offspring can arise in several ways. In response to clutch enlargement, parents may increase their own energy expenditure (Moreno *et al.* 1991) so that the energy invested per egg is kept unchanged. Attending parents may also be reluctant or unable to increase their own expenditure in reaction to clutch enlargement, and as a consequence reduce the investment per egg. Reduced investment may lead to poorer developmental conditions for embryos in enlarged clutches (Webb 1987). Alternatively, with clutch enlargement the thermal property of the clutch changes; eggs incubated in enlarged clutches experienced higher egg temperature, and lost more weight, probably because of these higher temperatures (Reid *et al.* 2000b). Higher egg

Table 3.1. Summarizing scheme with possible type of experiments to isolate different fitness effects of clutch size manipulation during incubation on post-hatching performance.

methodology		code		consequences			references	
		type of manipulation experiment	costs of incubation isolated	distinction of effects possible	counteracting of effects prevented	confounding effects excluded		
eggs not returned at hatching	control; unmanipulated during incubation phase	B ₁	no	-	-	-	Sanz 1997 Hanssen <i>et al.</i> 2003, 2005	
	control; rearing additional young in nestling phase	B ₂	yes	no	-	-	Visser & Lessells 2001 Heaney & Monaghan 1995	
eggs returned at hatching	young not matched to egg	ratio not constant	C	yes	no	no	-	Engstrand and Bryant 2002; Cichon 2000; Ilmonen <i>et al.</i> 2002; Heaney and Monaghan 1996; de Heij <i>et al.</i> 2006
		ratio constant	C	yes	no	yes	-	de Heij <i>et al.</i> 2006
	young matched to egg	ratio not constant	C	yes	yes	yes	no	Reid <i>et al.</i> 2000b
		ratio constant	C	yes	yes	yes	yes	this study

temperatures may negatively influence developmental conditions during the incubation period, and hence may affect pre-hatching performance of embryos. When concentrating on pre-hatching performance, disentangling the mechanism via which effects of clutch size manipulation acts is not possible.

Effect of clutch size manipulations on post-hatching performance of nestlings can arise in two ways; either by carry-over effects from detrimental developmental conditions that embryos experienced during incubation (hereafter referred to as *effects via eggs*) or by reduced parental provisioning during the nestling phase as a result of over investment during the incubation phase (*effects via parents*). In case of the experiments type B, these two ways cannot be distinguished, because parents and eggs received the same treatment during incubation. When eggs are returned at hatching (type C), however, the two ways through which clutch size manipulation can affect the performance on parents and offspring can in principle be separated.

Acknowledging these two pathways creates the possibility to disentangle them, which may give insight in the mechanism through which clutch enlargement reduces fitness. It also identifies a potential problem; effects via the two pathways may counteract each other, depending on the scheme according to which eggs are manipulated (Fig. 3.2). The problem of counteracting effects arises only when eggs are returned to their original clutch (type C). As a consequence of returning eggs to their original clutch, parents of one treatment category may receive eggs from different treatment categories (Fig. 3.2, scheme I-IV, after incubation). When parents of one treatment category receive proportionally more eggs of another category, the effects via eggs and via parents can counteract each other. For instance, when parents of the reduced treatment category receive proportionally more eggs that have been incubated in enlarged clutches (Fig. 3.2; scheme III), positive effects of clutch reduction (via parents) may be counteracted by the negative effects of clutch enlargement (via eggs). The potential counteracting effects via parents and via eggs are especially problematic when effects of clutch size manipulation on offspring performance are determined on the average performance of a clutch, regardless of whether all eggs within the clutch receive the same treatment during incubation (Table 3.1). This problem can be avoided by ensuring a constant proportion of eggs from each treatment in all nests, like presented in scheme

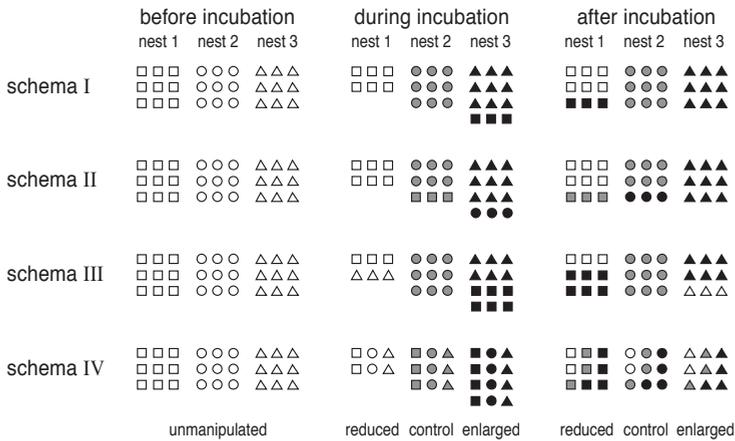


Figure 3.2. Manipulation schemes for exchanging eggs between nests within triplets; the composition of the nests are given before, during and after the incubation period. The shape of the symbols (square, circle and triangle) represents the nest of origin (1,2 and 3), while the colour of the symbols (white, grey and black) represents the manipulation category during incubation (reduced, control and enlarged). The words reduced, control and enlarged in the figure represent the treatment experienced by the parents associated with nest 1, 2 and 3, respectively. Most studies use method I (for overview of studies see de Heij *et al.* 2006, in press), method II used by Engstrand and Bryant (2002) and Moreno *et al.* (1991), method III is used by Smith (1989) and one year in chapter 2, and method IV is used in this study.

IV (Fig. 3.2); after restoring the original clutches, all parents end up with clutches that had similar treatment during incubation. If this scheme is used, the performance of a clutch can be determined using the average performance of all nestlings within the clutch.

The problem of counteracting effects via parents and via eggs does not exist when eggs are returned to their original clutch and nestlings are matched to the egg that they hatched from. If one knows what treatment individual eggs/nestlings received during incubation (Table 3.1), effects via eggs and via parents can be distinguished, since several combinations of parents and offspring exist. For instance, one can study whether nestlings with different treatment during incubation or whether nestlings reared by parents with different treatment during incubation perform differently. Again, keeping the ratio constant is to be preferred to achieve an orthogonal design (Fig 3.2., scheme IV), and hence enable the full separation of effects via parents and effects via eggs.

So far, one study has made an attempt to disentangle the two ways through which clutch size manipulations can effect post-hatching (Table 3.1). In a study on roof nesting starlings *Sturnus vulgaris* in Spain, Reid *et al.* (2000) determined pre-hatching performance of one egg that was incubated in an enlarged clutch relative to the performance of its siblings that were incubated in a control clutch. This study found that eggs incubated in enlarged clutches had a reduced hatching probability. Reid *et al.* (2000) could follow the fate of 9 of the 17 nestlings that hatched from eggs incubated in the enlarged clutch. They found no indication for increased mortality of these nestlings.

I performed clutch size manipulations during the incubation phase (type C) in two breeding seasons in a population of great tits (*Parus major* L.). Eggs were exchanged between nests following manipulation scheme IV. This scheme enabled me to use of a within-clutch comparison while studying the effects of clutch size manipulation on pre-hatching performance, because I could compare the performance of eggs with a given inherited egg quality (i.e. parental origin), but that had experienced different clutch sizes during incubation. Moreover, the use of this scheme enabled me to disentangle the effects via the two ways on post-hatching performance, since part of the eggs received a different treatment during incubation than their parents. To distinguish between the treatment experienced by parents and by their offspring, I will talk about *parental treatment* and *egg treatment*. At hatching, I was able to link about one-third of all hatchlings to the egg treatment, and therefore no full justice could be done to the design of the experiment. Nevertheless, in this study I will provide a first insight in how effects of clutch size manipulation via eggs and via parents can affect post-hatching performance of offspring. The effects of clutch size manipulation on offspring performance studied from the perspective of the parent and hence concerned with the average performance of all chicks within the nest are given and discussed in a previous paper (chapter 2).

METHODS

General procedure

This study was conducted in a nest-box breeding population of great tits (*Parus major*) in the woodlots of the Lauwersmeer, in the northern Netherlands (53°20'N, 06°12'E) in the years 2002 and 2003. From the beginning of April till July, nest-boxes were checked at least once a week to establish laying date and clutch size. From the sixth egg onwards, nests were visited daily to determine the onset of incubation. The onset of incubation was defined as the first day on which either the female was found incubating or the eggs were found uncovered and warm.

Clutch size manipulation

Clutch size manipulations were performed on the 2nd day of incubation and restored on the 11th day of incubation (for more details see chapter 2). Manipulations were performed in groups of three nests (i.e. triplets) that were matched for clutch size (maximum difference one egg) and the onset of incubation (no variation). Within each triplet, nests were randomly assigned to one of the treatments; reduced, control or enlarged. Clutches were reduced or enlarged by two (2002: 6 out of 19 triplets; 2003: all triplets) or three eggs (2002: 13 of 19 triplets; chapter 2).

At both the beginning and end of incubation clutches were manipulated and restored according to the same manipulation scheme. Manipulation scheme IV (Fig. 3.2) was used to exchange eggs between nests within triplets; eggs originating from the same nest were distributed over all three treatment categories. At the same time, parents received either a reduced, control or enlarged clutch to incubate. Eggs were transported in warmed insulated boxes. Transport of the eggs was kept to the minimum to reduce thermal stress (mean = 17 min; range 4 – 50 min). To keep track of the origin of the eggs, eggs were marked at the apex with a marker pen.

After turning the eggs to their original nests, nests were visited twice a day to link hatchlings to their egg of origin. Groups of newly born hatchlings were marked for identification by removing one or two pieces of down on their head and the symbols on the remaining eggs were noted. If newly born hatchlings belonged to eggs with different symbols, the hatchling could not be assigned to the egg of origin. In 2002 and 2003, respectively, 104 out of 332 (31.3 %) and 82 out of 240 (34.2%) of the hatchlings were linked to the egg of origin. For these nestlings the clutch size treatment during the incubation phase was known. The developmental time of hatchlings with known egg treatment did not differ in developmental time from the mean developmental time of that clutch (deviation in developmental time from zero; 2002: 0.10 ± 0.74 days, $n = 104$, t -test: $t_1 = 1.39$, $p = 0.17$; in 2003: 0.08 ± 0.56 days, $n = 82$, t -test: $t_1 = 1.37$, $p = 0.17$). When the oldest hatchling in the nest was 7 days old (day of hatching is 0), all nestlings were ringed and at 14 days their body mass (gram), wing length (mm)

and tarsus length (mm) were measured. Both body mass and wing length still changes at that age, but tarsus length is relatively fixed (Gebhardt-Henrich and van Noordwijk 1994). Tarsus length, therefore, provides a measure of the structural size of an individual later in life and reflects early growth conditions of the nestling. When nestlings were 18 days old, nests were visited every day to determine fledging date and the number of fledglings. Afterwards, nests were removed and checked for dead nestlings. The probability to recruit locally in the breeding population was estimated on the basis of recaptures of breeding birds in the study area in the subsequent year.

Response variables

Effects of clutch size manipulation were analysed on both pre-hatching performance and post-hatching performance of offspring. As measure of pre-hatching performance were used *development time* (time between first day of incubation and the day of hatching) and *hatching probability* (probability of an egg to hatch). As measure of post-hatching performance were used *body mass* (in gram), and the structural measures *wing length* (in mm) and *tarsus length* (in mm) of nestlings that were 14 days old. Additionally, the following fitness components were used as measure of post-hatching performance; *fledging probability* (probability of a hatchling to fledge) and *recruitment probability* (probability of a fledgling to recruit).

Statistical analysis

In total, 43 of 57 nests manipulated in 2002, and 36 of 48 nests manipulated in 2003 were used in the analyses. The other nests were excluded from the analysis for a variety of reasons, such as nest desertion (chapter 2) or missing values for developmental time.

When testing effects of clutch size treatment on pre-hatching performance of offspring, data analyses were performed using a general linear mixed-modelling approach with a hierarchy of nested effects using the program MLwiN 2.02 (Rasbash *et al.* 2000). Hierarchical levels were used to avoid pseudo replication when testing the effect of clutch size treatment on pre-hatching performance of offspring (eggs within a nest-box cannot be considered as independent data points). The following hierarchical levels were used (from highest to lowest level): 1) triplet, 2) nest within triplet (i.e. original nest-box with own parents) and 3) individual egg or nestling. The following explanatory variables were used: treatment (reduced, control or enlarged), year (2002, 2003), transport (whether eggs were transported or not), original clutch size (ranged from 6 to 12 eggs) and date of manipulation (ranged from April date 21 to 40).

When testing effects of clutch size treatment on post-hatching performance of offspring, preferably the interaction term between egg treatment and parental treatment is included in the model. However, because linking hatchlings to their egg of origin was possible in about 30% of the cases, sample size was limited and

the interaction between egg treatment and parental treatment could not be tested reliably. Consequently, egg treatment and parental treatment were included in the model without incorporating their interaction term. Furthermore, too few nestlings were available per nest for a proper within-clutch comparison. Therefore, nestlings with known egg treatment were included in the analyses as independent data points, and hence no hierarchical levels were taken into account. Given that the age of nestlings within clutches varied at the day of measurement, age is incorporated as a covariate in the statistical analysis on effects of egg treatment on nestlings' body mass and wing length. The following explanatory variables were used: egg treatment (reduced, control or enlarged), parental treatment (reduced, control or enlarged), year (2002, 2003) and original clutch size (ranged from 6 to 12 eggs). Each model was derived using backward elimination of possible explanatory variables and their two-way interaction terms. All tests were two-tailed: α is 0.05 was used as significance level. All values are presented as means \pm SD.

RESULTS

General

Laying date of all clutches involved in the experiment differed between the years ($\chi^2_1 = 30.7$, $p < 0.001$); females started laying on average one week later in 2003 as compared to 2002 (2002: 18.4 ± 5.1 , $n = 57$; 2003: 25.4 ± 3.0 , $n = 48$ – day 1 is the first of April). Clutch sizes also differed between years ($\chi^2_1 = 54.2$, $p < 0.001$); clutches were smaller in 2003 than in 2002. Clutches contained on average 9.2 ± 1.0 eggs ($n = 57$) in 2002 and 7.9 ± 1.2 eggs ($n = 48$) in 2003. In 2002, incubating females experienced on average lower ambient temperatures during the incubation phase (taken as running mean over mean daily ambient temperatures over the whole incubation period) than in 2003 (year effect, $\chi^2_1 = 6.9$, $p < 0.01$; Fig. 3.3). Clutches were more synchronized in 2003 as compared to 2002 (Fig. 3.3).

Pre-hatching performance

Developmental time of eggs incubated in control clutches was on average 12.9 ± 1.1 days and 12.3 ± 1.3 days in 2002 and 2003, respectively (Table 3.2; Fig. 3.4). In 2002, eggs that had been incubated in an enlarged clutch needed an additional 0.3 day to hatch relative to control; an effect that did not occur in the year 2003 (as is clear from the significant interaction term in Table 3.3, and Figure 3.4). Developmental time declined in the course of the season in both years; this correlation was approximately significant (Table 3.3).

Hatching probability of an egg was on average 0.92 ± 0.11 ($n = 79$; in Table 3.2 overview per manipulation category and year), and did not depend on egg treatment or year (Table 3.3). Also date of manipulation, transport and original clutch size did not explain variation in hatching probability (Table 3.3).

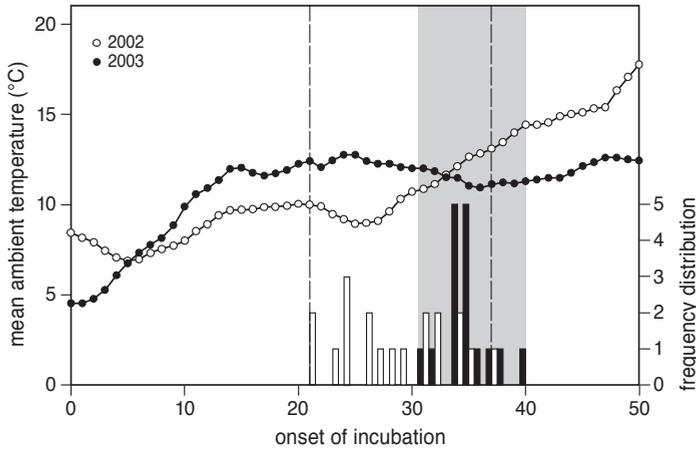


Figure 3.3. Running mean of ambient temperature during the incubation phase (assuming incubation period of 12 days) in relation to April date (1 = first of April) for clutches involved in the clutch size manipulation in the years 2002 and 2003. The area represents the range of dates between the first and the last manipulated clutches for that year. The bars represent the frequencies of triplets that were manipulated on the same day.

Table 3.2. Overview of offspring performance (mean \pm SD) per year and per treatment category.

	2002			2003		
	reduced	control	enlarged	reduced	control	enlarged
pre-hatching performance						
number of nests	12	17	14	10	12	14
developmental time	12.8 \pm 1.1	12.9 \pm 1.1	13.2 \pm 1.2	12.1 \pm 1.2	12.3 \pm 1.3	12.2 \pm 1.3
hatching probability	0.93 \pm 0.16	0.91 \pm 0.18	0.91 \pm 0.15	0.93 \pm 0.17	0.95 \pm 0.16	0.94 \pm 0.13
post-hatching performance						
number of individuals	20	40	30	16	25	29
body mass (gram)	16.4 \pm 1.8	16.0 \pm 1.7	15.6 \pm 2.4	16.4 \pm 1.8	16.3 \pm 1.6	16.1 \pm 1.6
tarsus length (mm)	19.7 \pm 0.5	19.5 \pm 0.6	19.2 \pm 0.8	19.9 \pm 0.6	19.9 \pm 1.0	19.5 \pm 0.6
wing length (mm)	32.8 \pm 0.7	32.3 \pm 0.6	29.0 \pm 0.9	30.2 \pm 0.8	30.3 \pm 0.9	31.3 \pm 0.7
number of individuals	23	45	36	19	29	34
fledging probability	0.96 \pm 0.21	0.96 \pm 0.21	0.89 \pm 0.32	0.95 \pm 0.23	0.93 \pm 0.26	0.94 \pm 0.24
recruitment probability	0.05 \pm 0.21	0.12 \pm 0.32	0.06 \pm 0.25	0.11 \pm 0.32	0.11 \pm 0.32	0.16 \pm 0.37

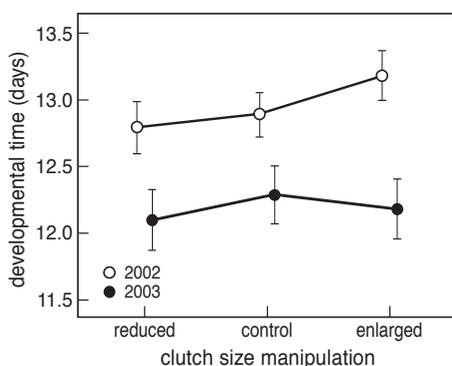


Figure 3.4. Developmental time (mean ± SE) in relation to egg treatment (i.e. the treatment that eggs received during incubation) for the years 2002 and 2003.

Table 3.3. Summaries of models that examined the within-clutch variation of pre-hatching performance.

final model		developmental time				hatching probability			
		B±SE	χ^2	df	p	B±SE	χ^2	df	p
year (A)	2002	12.85±0.20	3.6	1	0.06	1.59	1	0.21	
	2003	12.29±0.22							
manipulation (B)	reduced	-0.07±0.10	0.57	2	0.75	0.05	2	0.97	
	control	0							
	enlarged	-0.03±0.08							
A*B	2002 * reduced	-0.01±0.13	11.5	2	<0.01	0.30	2	0.68	
	2002 * control	0							
	2002 * enlarged	0.31±0.10							
rejected terms									
transport			1.50	1	0.22	1.31	1	0.25	
date manipulation		-0.07±0.04	3.32	1	0.07	0.03	1	0.86	
clutch size			0.58	1	0.45	0.11	1	0.74	
random effects –levels									
triplet		0.24±0.19				0.00±0.00			
nest-box (Triplet)		1.01±0.22				0.20±0.32			
individual egg		0.27±0.02				0.93±0.06			

Estimates of explanatory variables are provided when they significantly affected the dependent variable.

Post-hatching performance

Nestlings that hatched from eggs with different egg treatment differed in the length of their tarsi in both years ($\chi^2_2 = 11.0$, $p < 0.01$, when controlled for year and age; Fig. 3.5). In both years, nestlings hatched from eggs incubated in experimentally enlarged clutches had shorter tarsi than nestlings that hatched from eggs incubated in control clutches ($\chi^2_1 = 6.7$, $p < 0.01$), but nestlings with the reduced egg treatment had no longer tarsi ($\chi^2_1 = 0.8$, $p = 0.38$). Tarsus length was longer in 2003 than in 2002 ($\chi^2_1 = 4.6$, $p = 0.03$, when controlled for egg treatment). Parental treatment had no effect on tarsus length ($\chi^2_2 = 1.5$, $p = 0.48$; controlled for egg treatment and year).

In 2002, nestlings that hatched from eggs incubated in enlarged clutches were lighter and smaller (wing length) than nestlings that hatched from eggs incubated in either control or reduced clutches (Table 3.2). In this year, but not in 2003, these nestlings were also significantly younger than the rest of the young in the nest due to the longer developmental time (interaction term egg treatment · year; $\chi^2_2 = 9.1$, $p = 0.01$, when controlled for egg treatment and year). Body mass and wing length of nestlings were strongly correlated, (Pearson's correlation: 2002; 0.70, $p = 0.001$, body mass = 6.7 ([SE] 1.0) + 0.3 (0.03) · wing length; 2003; 0.51, $p = 0.001$, body mass = 9.5 (1.4) + 0.2 (0.04) · wing length); therefore, effects of clutch size manipulation are studied on body mass only. After correction for age, year and their interaction (interaction term; $\chi^2_2 = 3.8$, $p = 0.05$, when controlled for age and year), effects of egg treatment on body mass were no longer apparent ($\chi^2_2 = 0.9$, $p = 0.64$). Parental treatment did affect body mass; the effect approached significance ($\chi^2_2 = 5.8$, $p = 0.06$), whereby nestlings reared by parents of the reduced treatment category were heavier than nestlings reared by parents of both the control and enlarged treatment.

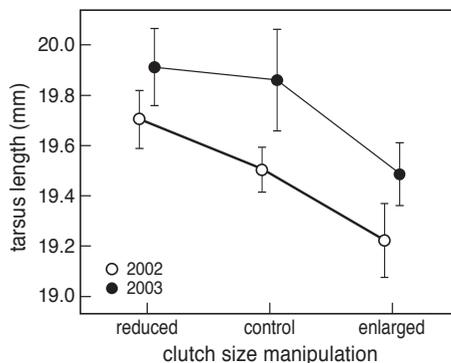


Figure 3.5. Tarsus length (mean \pm SE) in relation to egg treatment (i.e. the treatment that eggs received during incubation) for the years 2002 and 2003.

On average over all treatment categories and the two years, the probability for a hatchling to fledge was 0.94 ± 0.25 ($n = 186$), and the probability of a fledgling to recruit locally (i.e. found breeding in the same study population the subsequent year) was on average 0.10 ± 0.31 ($n = 174$; Table 3.2). Both fledging probability and local recruitment probability did not differ between the years ($\chi^2_1 = 0.1$, $p = 0.86$, and $v = 1.0$, $p = 0.31$, respectively). Neither egg treatment nor parental treatment had an effect on the probability to fledge (egg treatment: $\chi^2_2 = 0.8$, $p = 0.66$; parental treatment: $\chi^2_2 = 3.7$, $p = 0.16$), or the probability to recruit locally (egg treatment: $\chi^2_2 = 0.5$, $p = 0.80$; parental treatment: $\chi^2_2 = 0.5$, $p = 0.79$). Recruitment probability declined with original clutch size; this correlation approached significance ($B \pm SE$; 3.36 ± 0.19 , $\chi^2_1 = 3.4$, $p = 0.07$).

DISCUSSION

Using manipulation scheme IV, allowed for studying effects of clutch size manipulation on pre-hatching performance using a within-clutch comparison, and hence correct for inherited egg quality. Next, I could isolate effects of clutch size manipulation on post-hatching performance via eggs and via parents. Eggs incubated in experimentally enlarged clutches had longer developmental time in one of the two years, but had the same probability to hatch as eggs incubated in both reduced and control clutches. Independent of effects on developmental time, in both years nestlings that hatched from eggs incubated in enlarged clutches had shorter tarsi than those that hatched from eggs incubated in control or reduced clutches. Nevertheless, parental treatment had no effect on tarsus length. Fledging probability and recruitment probability did not differ between nestlings with different egg treatment.

Pre-hatching performance

Variation in environmental conditions, and consequently variation in the energetic costs of incubation, may explain why developmental time was not similar for the two years. As mentioned by previous studies, developmental time declined over the season (see also Smith 1989), and depends on year (see also Siikamäki 1995). These findings indicate that embryos can develop faster depending on the conditions during incubation, which are likely to be influenced by the incubation behaviour of the female. Apparently, females can incubate more intensively or for longer periods during the day, and thus improve developmental conditions of offspring of current clutches. Whether attending parents invest more in the current clutch should depend on the fitness costs of incubation and on the fitness value of the clutch. Later in the season, females are less likely to produce replacement clutches. Also later in the season, ambient temperatures are likely to be higher. In some years, birds may experience higher mean ambient temperatures during the

incubation period than in other years. Since ambient temperature strongly influences the energetic demands during incubation (Biebach 1984; Bryan and Bryant 1999; Weathers *et al.* 2002; chapter 6 and 7), and food availability (Perrins 1970), such may explain the lower fitness costs of incubation in the year 2003.

Clutch enlargement during incubation negatively affected developmental time in one of the two years, which suggests that these eggs experienced detrimental developmental conditions during incubation. Detrimental conditions may occur due to behavioural change of the female in reaction on clutch enlargement (Wiebe and Martin 2000) or due to change in thermal properties of the clutch with clutch enlargement (Reid *et al.* 2000b). A more detailed study on the physical property of clutches in relation to the number of eggs in the clutch, like done in the study by Reid *et al.* (2000b), are needed to unravel the effects via the two ways on pre-hatching performance.

In the same year that eggs incubated in enlarged clutches had longer developmental time than eggs in control clutches, eggs in reduced clutches had no shorter developmental time. With clutch enlargement, the energetic costs of incubation may increase (Moreno *et al.* 1991). Additionally, females incubating enlarged clutches may be physically unable to cover all eggs and incubate them efficiently. As a result, peripheral eggs cool even when the female is attending the clutch, leading to fluctuating egg temperature in enlarged clutches (Reid *et al.* 2000b) and hence prolonged developmental time. In both years, the average clutch size was above the upper limit of 7 eggs that female great tits are able to cover simultaneously (Mertens 1977). These options may explain why effects on developmental time are only found with clutch enlargement, but not with clutch reduction.

The use of within-clutch comparison to study effects of clutch size manipulation on pre-hatching performance allowed for the elimination of variation in quality between parents or territories/ nests at the time of egg laying. Although clutches are assumed to be attributed randomly to a certain treatment category, variation in quality between parents or territories may obscure the data, thereby making it harder to demonstrate effects. Comparison of the results of this study relative to that of others, however, does not reveal systematic differences. Three studies manipulated clutches during incubation, but did not restore them at hatching (Moreno and Carlson 1989; Smith 1989; Siikamäki 1995) (Fig. 3.1, B₁). Thus all eggs within a clutch received the same egg treatment, but may have belonged to different parents. In these studies eggs incubated in enlarged clutches had longer developmental time than eggs incubated in control clutches (Moreno and Carlson 1989; Smith 1989; Siikamäki 1995). Like in this study, Moreno and Carlson (1989) and Siikamäki (1995) found no difference in developmental time between eggs incubated in control or reduced clutches. Smith (1989) could not estimate the shape of the relationship between developmental time and clutch size, since he had either reduced or enlarged clutches. Only in the study by Engstrand and

Bryant (2002), in which manipulation type C (Fig. 3.1) and scheme number II (Fig. 3.2) was used, a linear relationship between developmental time and clutch size manipulation is found. In this case, however, effects found may possibly occur due to taking the average performance of all nestling within the clutch, while not all hatchlings received the same egg treatment.

Post-hatching performance

In both years, nestlings hatched from eggs incubated in enlarged clutches had shorter tarsi, when corrected for variation in age and year. Difference in tarsus length could not be explained by the treatment parents experienced during incubation. These results suggest that the effects of clutch enlargement on developmental conditions during incubation may carry-over to the nestling phase and thus may influence structural size of the nestlings later in life. The fact that effect on tarsus length was found in both years, while only in one year a clear effect was expected given the prolonged developmental time implies that developmental conditions may also affect post-hatching performance of embryos in a way other than through developmental time.

Like in the study of Reid *et al.* (2000b), nestlings hatched from eggs incubated in enlarged clutches did not suffer greater mortality during the nestling phase than nestlings hatched from eggs incubated in either reduced or control clutch. They also did not differ in the probability to recruit in the breeding population the following season. Yet, nestlings from eggs incubated in enlarged clutches had smaller tarsi. Birds with relatively larger tarsus length are found to have better access to food, and success in obtaining breeding territory (Drent 1983), and mate preference in males is found to be related to tarsus length (Blakey 1994, Kempenaers *et al.* 1992, Verboven and Mateman 1997). Therefore, I interpret the effect of egg treatment on tarsus length as indication that offspring with enlarged egg treatment have reduced fitness after fledging, although I did not find such an effect on local recruitment probability.

Parental treatment had also an effect on body mass of the nestlings, nestlings reared by parents with reduced treatment during incubation were heaviest. This implies that effect of clutch size manipulation on post-hatching performance not only occur via eggs, but also via parents. Thus, parents were affected by their treatment during incubation, when providing food to their offspring in the nestlings phase. This change in care, nevertheless, did not result in differences in fledging or recruitment probability. These findings are consistent with that of our previous study (chapter 2), in which the effect of the clutch size manipulation on post-hatching performance was determine from the perspective of the parents, and hence on the average of all nestling within the clutch.

In conclusion, the results of this study show that clutch size during incubation affects developmental time in one of the two years, which suggests detrimental developmental conditions during incubation. In both years, tarsus length of nest-

lings that hatched from eggs incubated in enlarged clutches was shorter, indicating fitness effects. Parental treatment had no effect on tarsus length, nevertheless, the lack of linking large number of hatchlings to their egg treatment resulted in a weakness in design. Depending on the scheme with which eggs are exchanged between nests, effects via eggs can be concealed, while this study indicates that these effects may be of importance in unravelling the mechanism through which clutch size affect fitness. Furthermore, the problem of counteracting effects which is in principle present in several studies turns out to be real and should be bared in mind for future work. The question whether nestlings with different egg treatment and subsequently reared by parents with different parental treatment during incubation perform differently remains unanswered in this study; the interaction term between egg treatment and parental treatment could not be reliably tested due to the low number of nestlings with known egg treatment. To be able to answer that question the probability to link each nestling to the egg of hatching should be increased, which can be done by visiting nests more frequent around hatching or by bringing the eggs prior to hatching from the field to the laboratory and hatch them in an incubator.

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