

# The costs of egg production and incubation in great tits (*Parus major*)

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The costs of egg production and incubation may have a crucial effect on avian reproductive decisions, such as clutch size and the timing of reproduction. We carried out a brood-size enlargement experiment on the great tit (*Parus major*), in which the birds had to lay and incubate extra eggs (*full costs*), only incubate extra eggs (*free eggs*) or did not pay any extra cost (*free chicks*) in obtaining a larger brood. We used female fitness (half the recruits produced plus female survival) as a fitness measure because it is the female which pays the costs of egg production and incubation, and because clutch size is under female control. Female fitness decreased with increasing costs (fitness of *free chicks* females is higher than that of *free eggs* females which is higher than that of *full costs* females). These fitness differences were due to differences in female survival rather than in the number of recruits produced. This is the first time that the costs of egg production and incubation have been estimated using such a complete fitness measure, including, as our measure does, the local survival to the following year of both the female and her offspring. Our results emphasize that reproductive decisions cannot be understood without taking egg production and incubation costs into account.

**Keywords:** fitness costs; clutch size; *Parus major*; egg production; incubation; climate change

## 1. INTRODUCTION

Two of the most intensely studied aspects of avian reproduction are timing of reproduction and clutch size (Lack 1947, 1968; Perrins 1970; Godfray *et al.* 1991). In studies of timing there has always been a strong emphasis on the idea that egg production may constrain the start of reproduction (Perrins 1970). However, studies of clutch size in birds which feed their young have often ignored the costs of egg production and incubation because these were assumed to be negligible compared with the costs of chick rearing (but see Lessells 1991). Consequently, many investigations of clutch-size manipulated the size of the family at or soon after hatching (Perrins & Moss 1975; Lessells 1986; Pettifor *et al.* 1988; Tinbergen & Daan 1990; Tinbergen & Both 1999). It was not until Monaghan and co-workers published a series of papers (Monaghan *et al.* 1995, 1998; Heany & Monaghan 1995; Monaghan & Nager 1997; Thomson *et al.* 1998; but see also Siikamäki 1995) that the effect of the costs of egg production and incubation on clutch size was widely discussed.

Recent studies of the energetic costs of egg production and incubation (daily energy expenditure) suggest that females may have to work equally hard during egg production, incubation and chick rearing (barn swallows *Hirundo rustica* (Ward 1996); great tits *Parus major* (Stevenson & Bryant 2000; Bryant & Bryant 1999; Tinbergen & Dietz 1994)). However, energetic costs may not reflect the fitness costs that ultimately affect the evolution of traits (Bryant 1988; Tinbergen & Verhulst 2000). Few experiments have attempted to measure the fitness costs of egg production (e.g. Monaghan *et al.* 1995), incubation (e.g. Nelson 1964; Moreno *et al.* 1991; Siikamäki 1995) or both (e.g. Heany & Monaghan 1995),

and even these use an incomplete measure of fitness (the number of chicks fledged).

The aim of this study was therefore to measure the fitness costs of egg production and incubation in the great tit (*P. major*) by comparing three experimental groups of females that had laid and incubated additional eggs, incubated additional eggs, or been given the same number of additional nestlings soon after hatching. We examined the effects of these treatments on female fitness (estimated as the sum of female survival and half the number of offspring recruiting locally the following year) because it is the female which pays these costs (only females incubate in great tits) and because clutch size is under female control. We also investigate whether there are costs in terms of laying date or clutch size in the following year (Lessells 1986; Gustafsson & Sutherland 1988).

## 2. METHODS

### (a) Field methodology

The experiment was carried out in 1998 and 1999 on great tits breeding in the Hoge Veluwe (The Netherlands, 52°05' N, 05°50' E). The study area consists of 171 ha (1 ha = 10<sup>4</sup> m<sup>2</sup>) of mixed woodland containing about 400 nest-boxes.

In 1998 and 1999, all nest-boxes were visited at least twice a week from the beginning of April onwards to monitor nest building, and were inspected daily once the nestcup became apparent. In all cases we found the first egg of a clutch on the day it was laid. When a first egg was found, the nest was attributed to the next experimental category given on a predetermined list. Successive groups of seven nests on the list contained the treatments hereafter known as *full costs* (twice), *free eggs* (once), *free chicks* (once), *control* (once) and *extra* (twice) in a sequence that was separately randomized within each group. As a result, laying date did not vary between the three experimental treatments

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(table 1). Some females were included in the experiment in both years but our procedure resulted in the experimental treatments being independent over the two years for each female.

The treatments were as follows:

- (i) *Full costs* pairs had to lay extra eggs, incubate them and raise the extra chicks. This was achieved by removing the first four eggs of the clutch on the day they were laid. We removed four eggs because we wanted females to lay two additional eggs, and Oppliger *et al.* (1996) had found that great tits laid one additional egg when they removed the first two eggs laid. The removed eggs were numbered with non-soluble marker pen, kept in a bed of moss at ambient temperature, and turned twice a day. The eggs were returned to the nest when a further five eggs had been laid. As a result, eggs were usually returned before any incubation so that the natural degree of hatching asynchrony was unaffected, but in some cases the female was found incubating before a further five eggs had been laid (this was mainly in 1999, when clutch sizes were relatively small) and the removed eggs were returned immediately.
- (ii) *Free eggs* pairs had to incubate and raise two extra eggs. Two eggs (marked with non-soluble marker pen) were added to the clutch on the day that incubation started (the female found incubating or the eggs found uncovered and warm). These two eggs were taken from an *extra* nest at which incubation started on the same day.
- (iii) *Free chicks* pairs had only to rear two extra chicks. These were added on day 2 (where day 0 is the day that the first chicks in the nest hatch) from *extra* clutches that had begun to hatch on the same date.
- (iv) *Control* pairs raised the same number of chicks as the number of eggs laid.
- (v) *Extra* clutches were used as donors for eggs or chicks. However, if no eggs or chicks had been removed by day 2, the brood was treated as either a *control* or a *free chicks* brood. (In addition, one *extra* nest was redesignated as a *free eggs* nest; see table 1.)

Clutches that were deserted at laying, incubation or at hatching were excluded from the analyses. The additional disturbance during laying for the *full costs* treatment did not affect the probability of desertion during laying (1998: *full costs*, 2 out of 26; *free eggs* and *free chicks*, 2 out of 33; 1999: *full costs*, 5 out of 28; *free eggs* and *free chicks*, 4 out of 36; ANOVA with binomial errors and year as a covariate:  $\chi^2_{(1)} = 0.59$ ;  $p = 0.44$ ).

All nests were visited daily during laying to record laying gaps and the start of incubation. After the start of incubation, nests were visited to read the colour-ring combinations of incubating females, and then daily (before 12.00 up to and including the hatching date) from a few days before the expected hatching date until day 2. Newly hatched chicks were individually marked by clipping one or two of the six tracts of down. On day 2, any unhatched eggs in *full costs*, *free eggs*, *free chicks* or *control* broods were moved into *extra* nests, and randomly chosen chicks from *extra* nests that had begun hatching on the same date were used to restore brood size to the clutch size laid in *full costs* and *control* broods, and the clutch size laid plus two in *free eggs* and *free chicks* broods. In four cases no chicks were available and these broods were therefore excluded from the experiment. Unhatched eggs were replaced in order to control for any effects on hatching success of transferring eggs between nests or storing eggs from *full costs* nests in artificial nests. The proportion of eggs failing to hatch was indeed higher in *full costs* and *free eggs*

clutches (12.1 (1.5 s.e.)%) than in *free chicks* and *control* clutches (8.1 (1.5 s.e.)%) (ANOVA with binomial errors:  $\chi^2_{(1)} = 5.11$ ;  $p = 0.024$ ). A within-clutch comparison (ANOVA with binomial errors) of the 32 clutches containing both marked (= transferred between nests) and unmarked eggs, and both hatched and unhatched eggs, and where it was known how many marked and unmarked eggs hatched, revealed that marked eggs had lower hatching success ( $\chi^2_{(1)} = 5.92$ ;  $p = 0.015$ ). When unmarked eggs were analysed separately, hatching success did not differ between eggs from *full costs* and *free eggs* clutches and from *free chicks* and *control* clutches ( $\chi^2_{(1)} = 0.75$ ;  $p = 0.75$ ), or vary with the clutch size incubated ( $\chi^2_{(1)} = 0.01$ ;  $p = 0.92$ ). These retrospective analyses show that the reduced hatching success of eggs in the *full costs* and *free eggs* treatment is a side-effect of marking, transferring or storing eggs, and justifies our procedure of replacing unhatched eggs with nestlings from *extra* broods.

All chicks were blood sampled (10  $\mu$ l from the leg vein) on day 4 to determine their sex using a reliable molecular technique (Griffiths *et al.* 1998) and ringed on day 7. The parents were caught on days 11 and 12 in 1998, and day 8 in 1999. Parents were identified from existing rings, and ringed and colour ringed as necessary. In 1998, a blood sample (maximum of 50  $\mu$ l) was taken from the brachial vein of all parents on day 11, in order to assess whether increased parental effort affected survival through *Plasmodium* or *Haemoproteus* infections. We found no *Plasmodium* and only one *Haemoproteus* (for a female of the *free eggs* treatment) in the blood smears of 32 females (5000 erythrocytes were scored per blood smear under  $\times 1000$  magnification) so we discontinued these measurements and did not take blood samples to assess parasite infections in 1999. In 1998, we also attempted to measure immune function by injecting 0.2 mg phytohaemagglutinin (Sigma L-8754, Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) in 0.04 ml phosphate-buffered saline intradermally in one of the wing webs on day 11 and measuring the increase in wing thickness between days 11 and 12 (Saino *et al.* 1997). This was discontinued after the first 107 adults because of difficulty in obtaining repeatable measurements.

In both years, the mass and tarsus length of the chicks were measured on day 15. After fledging, the nest was removed and the ring numbers of any dead chicks noted. Nest-boxes were checked weekly until mid-July in order to record any second broods (including identification of parents and ringing of chicks).

In 2000, all nest-boxes were checked at least weekly to determine laying date and clutch size, and catch and identify all breeding great tits. These data were used to determine the recruitment of chicks, and the overwinter survival, laying date and clutch size of parents of the 1999 experiment.

We estimated overwinter survival as the proportion of females that were recaptured in the year following the experiment. Capture-recapture analysis (Bauchau & Van Noordwijk 1995) shows that in our long-term study we typically catch 94% of the females that are alive. This figure does not include females that have emigrated permanently from the study area and therefore our survival estimate approximates local survival.

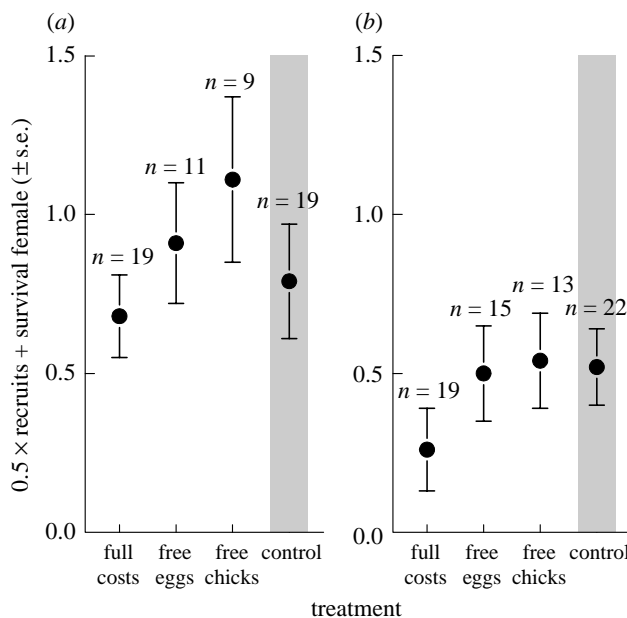
## (b) Statistical analysis

There is a clear directional hypothesis that the fitness of females should decrease in the order *free chicks*, *free eggs*, *full costs*, because all three groups of females attempted to raise the same number of chicks, but had to pay increasing costs of incubation, or egg production and incubation. We therefore tested the effect of these three treatments on fitness or fitness components using

Table 1. Mean(*s.e.*) laying date, clutch size and brood size at day 2 for the four treatment groups: control, full costs, free eggs and free chicks

(Abbreviation: n.a., not applicable.)

	1998						1999					
	treatment				ANOVA <sup>a</sup>		treatment				ANOVA <sup>a</sup>	
	<i>full costs</i>	<i>free eggs</i>	<i>free chicks</i>	<i>control</i>	$F_{2,39}$	<i>p</i>	<i>full costs</i>	<i>free eggs</i>	<i>free chicks</i>	<i>control</i>	$F_{2,44}$	<i>p</i>
sample size <sup>b</sup> (from <i>extra</i> )	21(0)	11(0)	10(0)	19(6)	n.a.	—	19(0)	15(1)	13(1)	22(9)	n.a.	—
laying date (1 = 1 April)	20.4(1.1)	20.2(1.3)	18.8(2.0)	19.9(1.2)	0.35	0.71	19.2(0.9)	19.1(1.2)	17.5(1.4)	18.4(0.9)	0.64	0.53
clutch size	11.8(0.4)	9.5(0.4)	10.4(0.5)	10.4(0.3)	7.99	0.001	9.8(0.3)	8.8(0.4)	8.9(0.4)	8.9(0.4)	2.53	0.09
brood size	11.8(0.4)	11.5(0.4)	12.4(0.5)	10.4(0.3)	0.89	0.42	9.8(0.3)	10.8(0.4)	10.9(0.4)	8.9(0.4)	3.31	0.046

<sup>a</sup> *F*-ratios are for the treatment effect between the three experimental treatments: *full costs*, *free eggs* and *free chicks*.<sup>b</sup> Sample sizes are the total sample size for each treatment group. The sample size in parentheses is the number of broods that were originally designated as *extra* broods, but were reallocated to other treatment groups (see § 2).Figure 1. Female fitness ( $0.5 \times$  number of recruits produced from both first and second broods + female survival) in relation to experimental treatments differing in the experimentally induced costs of egg production and incubation. Sample size for each treatment (*n*) is indicated above each standard error bar. (a) 1998, (b) 1999.

an ordered heterogeneity (OH) test (Rice & Gaines 1994). This takes into account both the *p*-value obtained from a non-directional heterogeneity test, and the Spearman's correlation coefficient ( $r_s$ ) for a trend across the treatment means in the predicted direction. For most response variables, the heterogeneity test underlying the OH test was an ANOVA with normal errors with year as a factor to account for year differences. For variables with a binary response (such as survival) ANOVAs with binomial errors and a logit link were used. In the analysis of number of recruits an ANOVA with Poisson errors was used. As a heterogeneity test underlying the OH test on female fitness a Kruskal–Wallis test was used on the residuals after fitting only year as a factor in an ANOVA with normal errors. To obtain  $r_s$ , the means of the residuals from an ANOVA with only year as a factor were correlated with treatment (in the

order *full costs*, *free eggs*, *free chicks*). All OH tests are one-tailed because we have a clear directional prediction of the effect of the treatment.

*Control* broods could not be included in the OH test because although the parents paid lower costs than the three experimental treatments, they also attempted to raise fewer chicks, so there was no clear *a priori* prediction as to their relative fitness. Instead, these broods were used to judge whether the natural clutch size could be regarded as optimal when the costs of raising chicks, incubating eggs and producing eggs were successively taken into consideration.

Throughout, all values are given with their standard errors in parentheses.

### 3. RESULTS

#### (a) *Experimental treatments*

In total, 130 great tit broods were included in the *control*, *full costs*, *free eggs* or *free chicks* treatments (table 1). The aim of the experimental protocol was for brood size on day 2 in the three experimental treatments (*full costs*, *free eggs* and *free chicks*) to be two chicks larger than the clutch size that the female would have naturally laid. Because females were randomly allocated to treatments this should mean that brood size on day 2 did not vary between the three experimental treatments. This was the case in 1998, but in 1999 brood size on day 2 varied between the three experimental treatments ( $p=0.046$ ), because the birds in the *full costs* treatment laid only 0.9 eggs more than the *control* birds (1.4 eggs in 1998; table 1). Fewer additional eggs were probably laid in 1999 than 1998 because they were more costly to produce: *control* clutches were 1.5 eggs smaller (8.9 versus 10.4) and temperature, which affects daily energy expenditure during laying (Stevenson & Bryant 2000), was 1.7 °C lower during egg production in 1999 (earliest to latest egg laying dates of *control* and experimental broods: 8–29 April; mean daily minimum temperature of 3.7 °C) than 1998 (7–25 April; mean daily minimum temperature of 5.4 °C). The implications of the reduced number of additional eggs laid by *full costs* females in 1999 is addressed in § 4.

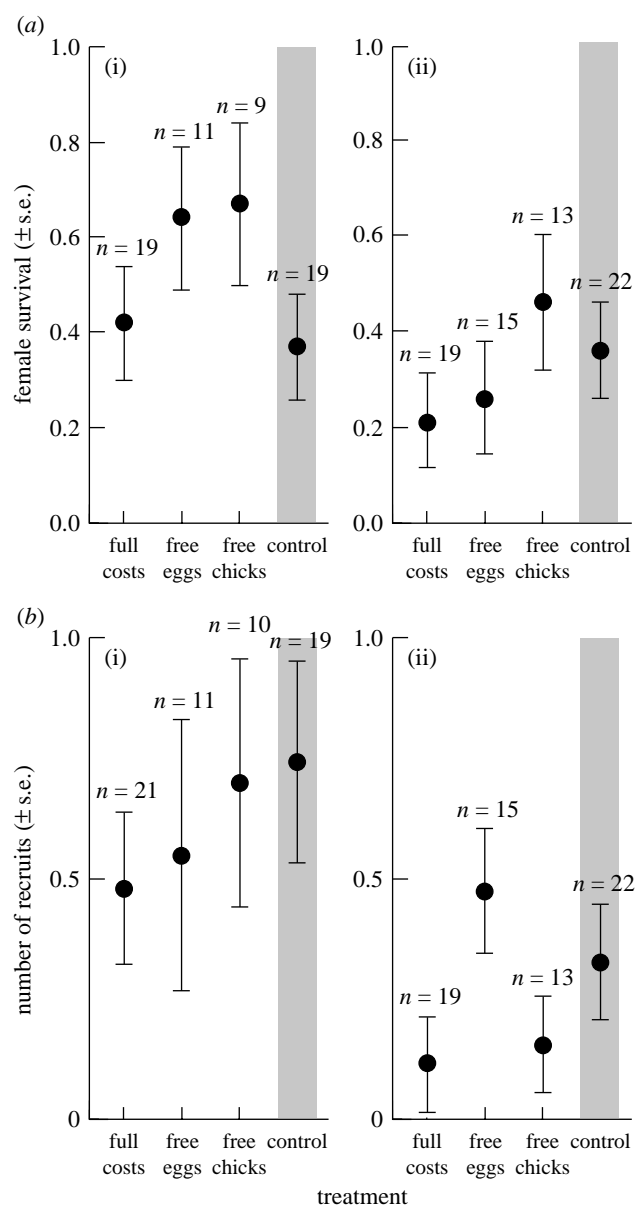


Figure 2. Two main components of female fitness (a) female survival, and (b) the number of recruits produced, in relation to experimental treatments differing in the experimentally induced costs of egg production and incubation. Sample size for each treatment ( $n$ ) is indicated above each standard error bar. (i) 1998, (ii) 1999.

#### (b) *Fitness costs of egg production and laying*

We assessed the fitness costs of producing and incubating additional eggs using the number of copies of the female's genes in the following year's breeding population as our fitness measure ( $0.5 \times$  number of locally recruited offspring (from both first and second broods) + female survival). Three broods where we did not identify the female were excluded from this analysis. Female fitness is clearly affected by the experimental treatment (figure 1; OH test;  $p=0.02$ ). Hence, there are clear fitness costs to the female of producing and incubating additional eggs.

To further explore these fitness costs we tested various fitness components. Female survival is clearly affected by experimental treatment: *full costs* females have the lowest, and *free chicks* females the highest, survival (figure 2a and table 2). The second major component of female fitness,

the number of recruits produced, was not significantly affected by treatment (figure 2b and table 2). The effect of treatment on this fitness component can be further broken down. There was no effect of treatment on the number of chicks fledged, the probability that these fledglings recruited, or the probability of producing a second brood (table 2). Recruitment probability itself depends on the condition of the fledglings (Verboven & Visser 1998); there was no effect of experimental treatment on fledgling quality (chick mass or tarsus length, whether or not corrected for chick sex; table 2).

Overall fitness may also be affected by the duration of incubation, which has been shown to be affected by the number of eggs incubated (Thomson *et al.* 1998). However, no effect of experimental treatment on incubation period was found (table 2). Including the number of incubated eggs in the analysis did not alter this conclusion (the number of incubated eggs itself was also not significant;  $p=0.15$ ). Hatching success was also unaffected by the clutch size incubated (see § 2).

Our fitness measure takes into account any effects on the probability of females or their offspring breeding in the following year, but the female's reproductive output in future years may also be affected. Among the 31 females that bred in the year following experimental manipulation, there was no effect of experimental treatment on clutch size (excluding the eight females from the 1998 experiment which were included in the 1999 *full costs* treatment), but *free chicks* females laid earlier, and *full costs* females later, than *free eggs* females (table 2).

Finally, we used female fitness as our fitness measure because the clutch size is under female control. Fitness costs to the male of increased clutch size should not affect the female's optimal clutch size, unless these also affect the female's own future reproductive prospects (cf. Lessells & Parker 1999). Nevertheless, the experimentally induced additional costs of egg production and incubation may affect the female's chick-provisioning effort and males may compensate for this (but see Sanz *et al.* 2000). Hence, there could be an effect of treatment on male survival. For the two years combined, we do not find such an effect (table 2), but the 1998 results show a strong effect in the predicted direction (OH test;  $p=0.005$ ).

#### (c) *Fitness costs of egg production and incubation, and optimal clutch size*

The relative fitness of *full costs*, *free eggs* and *free chicks* females allows the fitness costs of egg production and incubation to be estimated. The fitness of females from these three experimental treatments can also be compared with that of *control* females in order to identify the fitness costs that are sufficient to account for the observed clutch size in the population. Both *free chicks* and *free eggs* females have higher estimated fitness than *control* females (figure 3). Thus optimal clutch size is larger than the observed clutch size when only the fitness costs of incubating additional eggs and raising extra young is taken into account. However, *full costs* females have lower estimated fitness than *control* females: when the costs of egg production are taken into account as well, the observed clutch size is optimal (at least in comparison with an increase in clutch size of two eggs).

Table 2. *Fitness components (mean(s.e.)) in relation to experimental treatments differing in the experimentally induced costs of egg production and incubation*

	1998				1999				OH test $p^a$
	<i>full costs</i>	<i>free eggs</i>	<i>free chicks</i>	<i>control</i>	<i>full costs</i>	<i>free eggs</i>	<i>free chicks</i>	<i>control</i>	
female survival	0.42(0.12)	0.64(0.15)	0.67(0.17)	0.37(0.11)	0.21(0.10)	0.27(0.12)	0.46(0.14)	0.36(0.10)	0.025
number of recruits	0.48(0.16)	0.55(0.28)	0.70(0.26)	0.74(0.21)	0.11(0.11)	0.47(0.13)	0.15(0.10)	0.32(0.12)	0.25
number of fledglings	11.0(0.60)	10.9(0.40)	11.6(0.50)	9.1(0.60)	8.9(0.30)	9.5(0.70)	9.6(0.65)	7.7(0.50)	0.08
recruitment probability	0.045(0.015)	0.05(0.025)	0.07(0.025)	0.08(0.02)	0.01(0.01)	0.05(0.015)	0.015(0.01)	0.04(0.015)	0.25
mean chick mass (g)	17.1(0.29)	17.4(0.25)	17.2(0.15)	17.2(0.46)	17.1(0.33)	16.7(0.37)	17.1(0.34)	17.4(0.31)	0.45
mean chick mass (g) (corrected for sex <sup>b</sup> )	17.5(0.28)	17.9(0.27)	17.7(0.15)	17.6(0.43)	17.5(0.31)	17.2(0.38)	17.5(0.33)	17.9(0.31)	0.45
mean chick tarsus (mm)	19.6(0.09)	19.7(0.10)	19.6(0.07)	19.7(0.09)	19.6(0.10)	19.4(0.12)	19.5(0.10)	19.7(0.08)	0.50
mean chick tarsus (mm) (corrected for sex <sup>b</sup> )	19.9(0.09)	20.0(0.09)	19.9(0.07)	20.0(0.07)	19.9(0.10)	19.8(0.12)	19.8(0.10)	20.0(0.08)	0.55
incubation period (days)	13.1(0.14)	13.6(0.37)	12.5(0.27)	13.4(0.18)	13.4(0.19)	13.2(0.31)	13.5(0.24)	13.6(0.19)	0.70
probability of second brood	0.16(0.08)	0.09(0.09)	0.11(0.11)	0.11(0.07)	0(0)	0(0)	0(0)	0.10(0.07)	0.50
clutch size following year	9.7(0.76)	8.7(0.33)	9.2(0.58)	9.0(1.22)	10.2(0.63)	10.0(0.41)	9.2(0.48)	9.1(0.69)	0.96
laying date following year (1 = 1 April)	21.7(1.16)	18.3(1.13)	17.3(3.37)	17.7(1.60)	16.5(1.32)	16.0(1.32)	15.7(1.52)	16.6(2.22)	0.025
male survival	0.37(0.11)	0.55(0.16)	0.80(0.13)	0.29(0.11)	0.22(0.10)	0.23(0.12)	0.15(0.10)	0.37(0.11)	0.07

<sup>a</sup>  $p$ -values are for one-tailed OH tests (Rice & Gaines 1994) testing the directional hypothesis that the fitness components vary between treatments as *full costs*  $\leq$  *free eggs*  $\leq$  *free chicks* (except for laying date where the hypothesis tested is *full costs*  $\geq$  *free eggs*  $\geq$  *free chicks*).

<sup>b</sup> Based on all fledglings in 1998 and 1999 ( $n = 1316$ ) male fledglings are 0.93 g heavier and have 0.62 mm longer tarsi than female fledglings (ANOVA;  $p < 0.001$  for both variables. Year and year  $\times$  sex were not significant for both variables;  $p > 0.65$ ). Mean brood chick weights and tarsus lengths were therefore corrected for variation in brood sex ratio by adding 0.93 g to the weight and 0.62 mm to the tarsus length of female fledglings.

#### 4. DISCUSSION

Our experiment shows that the production and incubation of additional eggs has a clear effect on female fitness, mainly through a reduction in female survival rather than in the number of recruits produced.

Our experimental design aimed at producing three experimental groups of females, each raising a brood two chicks larger than the number of eggs the females would naturally have laid. In the *full costs* treatment we had limited control over the number of additional eggs produced by the females, and in 1999 *full costs* females raised smaller broods than *free eggs* and *free chicks* females. We want to stress that this cannot explain our results. The fitness cost of producing additional eggs is expressed mainly through reduced female survival: there were no significant differences between experimental treatments in the number of fledglings produced. The birds in the *full costs* treatment started off with fewer hatchlings, but did not fledge fewer chicks. If the *full costs* females had indeed laid two additional eggs, the reduction in female survival would presumably have been at least as severe, and their fitness the same or even lower.

In addition to the fitness costs included in our measure of female fitness, experimental treatment affected the laying date of surviving females in the following year. Although the size of the effect appears small (a difference of about three days between the *full costs* and *free chicks* females), this results in a relatively large estimated loss in female fitness of about 0.15 gene copies in the following year (slope of female fitness in relation to laying date in

an ANCOVA with year as a factor of *control* broods in 1998 and 1999 equals 0.05;  $p = 0.01$ ). Our measure of female fitness therefore underestimates, if anything, the fitness costs of egg production and incubation.

Optimal clutch size must be determined evolutionarily by some kind of fitness cost to the offspring or parents that increases with clutch or brood size. The obviously increasing need for parental provisioning of larger broods led early researchers seeking adaptive explanations of clutch size to concentrate on the nestling phase (e.g. Lack 1947). As a result, manipulation of brood size soon after hatching became a standard method of assessing the costs associated with large family size. If we had carried out only this manipulation (the *free chicks* treatment) in our experiment, we would have concluded that females with larger clutches were fitter than *control* females (figure 3). Only when the costs of egg production and incubation are included (the *full costs* treatment), is the estimated fitness of females with an enlarged family less than that of *controls* (figure 3). These costs are therefore essential to an adaptive explanation of clutch size in our population. Tinbergen & Daan (1990) and Tinbergen & Both (1999) had previously carried out extensive brood-size manipulations on the same population of great tits as used in our experiment. These studies suggest that experimentally increasing brood size soon after hatching leads to a reduction in female fitness, so that there is no need to invoke costs of egg production or incubation to explain the observed clutch size. Possible explanations for the difference between conclusions by us and their conclusions include annual variation in the fitness consequences of

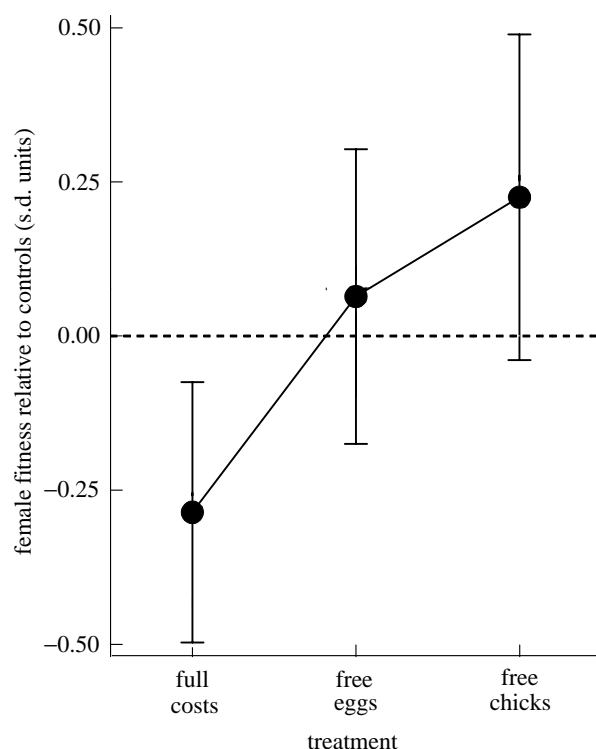


Figure 3. Optimal clutch size and the fitness costs of egg production and incubation. Values shown are the female fitness of experimental treatments raising an enlarged brood relative to that of *control* females. Experimental treatments differed in the experimentally induced costs of egg production and incubation. Plotted values are (mean treatment value – mean *control* value)/(s.d. of *control* values), where treatment and *control* values are the residuals in an ANOVA with year as the only factor. Error bars are the standard error of the difference between the mean treatment value and the mean *control* value ( $= ((\text{s.e. treatment values})^2 + (\text{s.e. control values})^2)^{0.5}$ , also expressed in units of s.d. of the *control* values). This measure of relative fitness was used in order to allow comparison with Monaghan & Nager (1997). Standard deviation of *control* values is 0.65; s.d.s of the treatment values are 0.56, 0.60 and 0.65, respectively.

brood-size manipulation (as exhibited within Tinbergen's results) or to the larger experimental increase in brood size in Tinbergen's study than in ours (J. M. Tinbergen, personal communication). Across different species, the existence of undetected costs of egg production and incubation may reconcile the mixed results of tests of optimal clutch size using brood-size manipulations (for a review, see Tinbergen & Both 1999). It may also explain why smaller than optimal clutch sizes are found in years with a low density of breeding pairs (Both *et al.* 2000). Conclusions about the adaptive nature of clutch size based on brood-size manipulation experiments should clearly be treated with caution.

If the costs of egg production are important in determining optimal clutch size, any phenotypic plasticity in clutch size might be expected to reflect in part conditions at the time of laying. Our results suggest that small clutch size might be associated with lower willingness to take on additional costs of egg production, and that ambient temperature might be an important environmental variable. An increase in daily energy expenditure during

laying with decreasing ambient temperature provides a possible mechanism by which this could occur (Stevenson & Bryant 2000). A similar link between environmental conditions at the time of laying and clutch size is revealed by food-supplementation experiments before and during laying (Boutin 1990; Nager *et al.* 1997). Rather few of these experiments have produced a response in clutch size (perhaps partly because small passerines tend to be more protein than energy limited during laying (Meijer & Drent 1999) and many experiments have provided seeds rather than a protein source), but those that have done so were carried out in years when population clutch size was low. If optimal clutch size is determined only by costs incurred during the nestling phase, we would not expect clutch size to be sensitive to conditions at the time of laying, unless these foretell conditions later in the season.

If the fitness costs of egg production are dependent on conditions at the time of laying, clutch-size decisions become inextricably linked with decisions on the timing of reproduction. Females might avoid high costs of egg production that reduce optimal clutch size by delaying the start of laying, but this may incur other costs, because this might destroy the synchrony between rearing of nestlings and the short peak in caterpillar abundance (Nilsson 1994; Verboven & Visser 1998). Conversely, the fitness costs of egg production that we have demonstrated may prevent females from laying earlier even if they are selected to do so by conditions during chick rearing. This is illustrated by the recent discussion on the effects of large-scale climate change (Visser *et al.* 1998). In our study area, higher spring temperatures have advanced the peak in caterpillar abundance, but not in laying date. One explanation for this is that temperatures in early spring have increased less than those in later spring (affecting caterpillar growth), and the high costs of egg production at low temperatures constrain females from laying earlier (Visser *et al.* 1998). This explanation is not at variance with the temporal increase in selection for early laying demonstrated by Visser *et al.* (1998), because the selection differentials that they calculated did not include the costs of egg production on female survival. Experiments manipulating laying date are needed to quantify the costs of egg production in relation to laying date before we can predict the optimal shift in laying date in response to increasing spring temperatures.

In conclusion, our experiments show that the fitness costs of egg production are not negligible in terms of their effect on optimal clutch size. This result sounds a warning note for the interpretation of brood-size manipulation experiments. Recognizing that egg production incurs fitness costs that may be dependent on environmental conditions at the time of laying is also essential to a complete understanding of the evolutionary response to large-scale climate change.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.