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Fitness cost of incubation in great tits (*Parus major*) is related to clutch size

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Life-history theory predicts that parents produce the number of offspring that maximizes their fitness. In birds, natural selection on parental decisions regarding clutch size may act during egg laying, incubation or nestling phase. To study the fitness consequences of clutch size during the incubation phase, we manipulated the clutch sizes during this phase only in three breeding seasons and measured the fitness consequences on the short and the long term. Clutch enlargement did not affect the offspring fitness of the manipulated first clutches, but fledging probability of the subsequent clutch in the same season was reduced. Parents incubating enlarged first clutches provided adequate care for the offspring of their first clutches during the nestling phase, but paid the price when caring for the offspring of their second clutch. Parents that incubated enlarged first clutches had lower local survival in the 2 years when the population had a relatively high production of second clutches, but not in the third year when there was a very low production of second clutches. During these 2 years, the costs of incubation were strong enough to change positive selection, as established by brood size manipulations in this study population, into stabilizing selection through the negative effect of incubation on parental fitness.

Keywords: clutch size manipulation; costs of reproduction; fitness consequences; life history; temporal variation

1. INTRODUCTION

Life-history theory predicts that parents produce the number of offspring that maximizes their fitness (Roff 1992; Stearns 1992). As resources are generally limited, they need to distribute their effort over various conflicting activities. Hence, parents need to trade-off their investment in the current breeding attempt with self-maintenance, and thus in potential future reproduction (Williams 1966; Charnov & Krebs 1974).

In birds, clutch size decisions have been studied intensively (Dijkstra *et al.* 1990; Lessells 1991; Vanderwerf 1992). The costs of reproduction are most commonly estimated by manipulating the number of young (i.e. brood size) and measuring the fitness consequences, as the ability of parents to provide food for the nestlings is generally considered to limit the clutch size. Life-history theory predicts stabilizing selection and expects both brood reduction and enlargement to result in lower fitness. Several studies indeed support this prediction (Gustafsson & Sutherland 1988; Lindén 1990; Tinbergen & Daan 1990); others, however, found negative (Verhulst 1995) or positive selection pressures (Tinbergen & Sanz 2004). Several reasons have been suggested as to why these experiments lead to opposite conclusions even though performed on the same species (see discussion section in Tinbergen & Sanz (2004)).

One of the potential explanations—the one we will concentrate on here—is that selection on clutch size does not only act during the nestling phase, but also during other phases in the reproductive cycle, such as the incubation phase (Monaghan & Nager 1997;

Visser & Lessells 2001). Since the costs were assumed to be negligible compared to that of rearing offspring, the incubation phase has long been ignored in studies on clutch size decisions (but see Lessells 1991). It is not until the 1990s that the costs of incubation became acknowledged (Heaney & Monaghan 1995, 1996; Monaghan & Nager 1997).

Since then, several studies have performed clutch size manipulations during the incubation phase (appendix A). A number of these studies show costs of incubation for offspring from enlarged clutches in terms of reduced hatching probability (Moreno *et al.* 1991; Siikamäki 1995; Reid *et al.* 2000b; Engstrand & Bryant 2002) or reduced fledging probability (Sanz 1997; Reid *et al.* 2000a). Yet the majority of studies performed their experiment during one breeding season and consequently measured fitness consequences in the short term (i.e. within that breeding attempt; appendix A).

Few studies measured fitness consequences in the long term (i.e. subsequent breeding attempts; appendix A). Two of these studies found a strong indication for reduced survival (Visser & Lessells 2001) and reduced fecundity in the subsequent breeding season (Hanssen *et al.* 2005) for females incubating enlarged clutches, and two other studies found no such costs (Sanz 1997; Hanssen *et al.* 2003, 2005; appendix A), however, cannot separate the costs of incubation from those of rearing offspring and thus do not indisputably identify the costs of incubation (for discussion see review by Reid *et al.* (2002)). To conclusively demonstrate these costs, more clutch size manipulations limited to the incubation phase are required.

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We manipulated clutch sizes during the incubation phase only during three breeding seasons and measured the fitness consequences for offspring and parents in both the short and the long term. In the population of great tits *Parus major* that we studied, we have evidence for a positive selection on clutch size during the nestling phase as revealed by brood size manipulations (Tinbergen & Sanz 2004). A fitness cost related to clutch size during the incubation phase might counteract this directional selection.

2. MATERIAL AND METHODS

(a) *Study population*

This study was conducted in the woodlots of the Lauwersmeer in the north of the Netherlands (53°20' N, 06°12' E) during the period 2000–2004. In this study period, about 200 nest-boxes were available in eight woodlots of different size (6–106 ha) interspersed with non-breeding habitat. For further details see Tinbergen (2005).

In great tits, the female incubates the eggs and the male may assist by feeding her. After eggs hatch, both parents invest in feeding the chicks. In this study population, clutches contained on average 9.3 ± 1.8 eggs ($n=1140$; 1994–2003). Some of the females (9–51%; 1994–2003) produced a second clutch after successfully rearing the first clutch.

(b) *Standard procedure*

From the beginning of April, nest-boxes were checked weekly to determine the laying date and clutch size. From the sixth egg onwards, nests were visited daily to determine the onset of incubation; either the female was found incubating or the eggs were found uncovered and warm. During first clutches, almost all females started incubation after clutch completion (94% of the manipulated clutches in the 3 years), but some individuals initiated incubation before all eggs were produced. Clutches were manipulated on the 2nd day and restored on the 11th day of incubation (i.e. just before hatching). Nests were checked daily around the expected hatch date to determine the number of hatchlings. When nestlings were 7 days old, both parents and nestlings were caught and ringed (for further details see Tinbergen & Sanz (2004)). When nestlings were 18 days old, nests were visited daily to determine fledging date and the number of fledglings. Afterwards, the nests were removed and checked for dead chicks. Further weekly checks allowed us to determine the incidence of second clutches and their breeding success. The identity of females caring for second clutches was in the majority of cases determined by recaptures and otherwise by reading colour rings during incubation; this information was used to ascribe second clutches to females caring for first clutches. Local survival probability of the parents and local number of recruits were estimated on the basis of recaptures of breeding birds in the study area in the subsequent year.

(c) *Clutch size manipulation*

Clutch size manipulations were performed on first clutches during the breeding seasons of 2000, 2002 and 2003. No clutch size manipulations were performed in 2001, since the study area was closed for the first weeks during that breeding season to prevent further breakout of foot and mouth disease.

Manipulations were performed in triplets, matched for clutch size (maximum difference one egg) and day of incubation (no variation) to minimize variation in environmental conditions and parental quality among treatment

categories. Within each triplet, nests were randomly assigned to a treatment category: reduced, control or enlarged. In 2000 (all triplets) and 2002 (13 out of 19 triplets), clutches were reduced and enlarged by three eggs. With this manipulation size, we adhered to previous studies on brood size manipulations in tit species to facilitate comparison (Smith 1989; Rytönen & Orell 2001; Tinbergen & Sanz 2004). During 2002 (6 out of 19 triplets) and 2003 (all triplets), clutches were reduced and enlarged by two eggs to reduce nest desertion (see later).

At both the beginning and end of incubation, clutches were manipulated and restored according to the same protocol. Following the method of Smith (1989) in 2000, two-third of the eggs in the reduced clutch were transported to the enlarged clutch, while one-third of the eggs in the latter clutch were transported to the reduced clutch. In the control clutch, half of the eggs were transported and returned to the same nest. In 2002 and 2003, we used a different setup. Eggs originating from one nest were incubated in all three nests of the triplet. After restoring the clutch, nests within triplets contained eggs that received on average the same treatment during incubation. Therefore, we can test the effect of clutch size manipulation on offspring fitness without correcting for the effects of the manipulation on the individual egg. Eggs within clutches that experienced different clutch size manipulation during incubation had similar hatching probability; neither fledging probability nor recruitment probability of young with known egg history did differ (details will be published elsewhere).

The amount of disturbance during the manipulation was minimized and was similar for parents of the three treatment categories. Eggs were transported in warmed insulated boxes to reduce thermal stress. Transport took on average 17 min (range 4–50 min). To prevent clutch desertion during egg transfer, the eggs were temporarily replaced by dummy eggs. To keep track of the origin of the eggs, the eggs were marked at the apex with a marker pen.

In total, 42, 57 and 48 nests were manipulated in the breeding seasons of 2000, 2002 and 2003, respectively. Original clutch sizes of manipulated nests ranged from 6 to 12 eggs; 80% of them were within the range of 8–10 eggs. Nests did not significantly differ in original clutch size ($\chi^2_2 = 1.5$, $p=0.46$, controlled for year) and onset of incubation ($\chi^2_2 = 3.6$, $p=0.17$, controlled for year) between manipulation categories. Nevertheless, years differed in original clutch size ($\chi^2_2 = 20.6$, $p<0.001$) and onset of incubation ($\chi^2_2 = 38.1$, $p<0.001$). In 2003, birds laid smaller clutches and started incubation one week later. Not all clutch size manipulations were successful; parents in the reduced treatment category were more likely to abandon their clutch immediately after manipulation (reduced 25%; control 0%; enlarged 4%; $\chi^2_2 = 21.2$, $p<0.01$; corrected for year; see §4).

(d) *The fitness components*

For first and second clutches, we analysed on a per nest basis the *hatching probability* (probability of a chick to hatch from an egg), the probability that a nest was successful (at least one chick fledged), the *fledging probability* (probability of a chick to fledge given that it hatched) and the *local recruitment probability* (probability of a chick to recruit locally given that it fledged). Additionally, we analysed the *probability of producing a second clutch*, and the *size* of the second clutches. These components were integrated in the number of recruits per first and second clutch and compared between manipulation categories.

Furthermore, we analysed the local parental *survival* (the number of adults breeding in the study area in the following breeding season for those nests of which we identified both parents). Some individuals had their clutches manipulated in more than 1 year (19 out of the 238 individuals—both males and females) and these were included in the analysis. We expected the survival effects of manipulation to occur in the first year after manipulation. Assuming that laying date and clutch size are under female-control, we analysed the *fecundity of the female in the subsequent season* (clutch size and laying date of a breeding bird in the subsequent season relative to the clutch size and laying date in the year of manipulation).

(e) *Statistical analysis*

The fitness components were analysed using a general linear mixed-modelling approach with a hierarchy of nested effects using the program MLwiN v. 2.02 (Rasbash *et al.* 2000). For first clutches, we used two levels (from highest to lowest level): (i) triplet and (ii) nest-box within triplet, while for local parental survival, we used three levels: (i) triplet, (ii) nest-box and (iii) individual (female and male) to account for the dependency between females and males within a pair. Since not all manipulated pairs produced a second clutch (see §3), clutches were not nested within the triplet in the analysis of second clutches. Explanatory variables were: experimental treatment (reduced, control or enlarged), sex (for parents), year and their interaction. Each model was derived using backward elimination of possible explanatory variables and the interaction terms. All values are presented as means \pm s.d., and all tests are two-tailed; *p*-values less than 0.05 were considered statistically significant (test results of interaction terms are not given if not significant).

3. RESULTS

(a) *First clutches*

In first clutches, hatching probability was 0.91 ± 0.11 ($n=119$). The probability that a nest was successful was 0.97 ± 0.18 ($n=119$), while the probability to fledge was on average 0.90 ± 0.15 ($n=115$; descriptive statistics summarized by year are given in table 1). Neither hatching probability differed between treatment categories within year ($\chi^2_2 = 3.44$, $p=0.18$) or between years ($\chi^2_2 = 2.28$, $p=0.32$), nor fledging probability (treatment effect, $\chi^2_2 = 0.58$, $p=0.75$; year effect, $\chi^2_2 = 1.38$, $p=0.50$). The probability to recruit the next year into the breeding population did not differ between treatment categories ($\chi^2_2 = 2.04$, $p=0.36$, controlled for year), but differed between years ($\chi^2_2 = 8.55$, $p<0.02$). For young raised in 2000, local recruitment probability was about half of that in the other two years (table 1). The number of recruits per first clutch in the subsequent breeding season did not differ between manipulation categories ($\chi^2_2 = 1.45$, $p=0.48$, controlled for year), but differed between years ($\chi^2_2 = 7.20$, $p<0.03$).

(b) *Second clutches*

Of the pairs manipulated in 2000 and 2002, 40% and 46%, respectively, produced a second clutch in contrast to 3% in 2003 (descriptive statistics summarized by year and manipulation categories are given in table 1). The last year was excluded from further analysis of the second clutches. The remaining second clutches contained on average 7.4 ± 1.0 eggs ($n=35$) and were smaller than first clutches

(paired *t*-test: $t_{34}=9.23$, $p<0.001$). Neither the probability to produce a second clutch differed with treatment (treatment effect, $\chi^2_2 = 2.05$, $p=0.36$, controlled for year; year effect, $\chi^2_1 = 8.32$, $p<0.02$), nor the size of the second clutches (treatment effect, $\chi^2_2 = 2.59$, $p=0.27$; year effect, $\chi^2_1 = 1.25$, $p=0.26$). Hatching probability of second clutches did not depend on the manipulation during first clutches ($\chi^2_2 = 3.22$, $p=0.20$, controlled for year), but differed between years ($\chi^2_1 = 9.52$, $p<0.002$), being lower in 2000. Nest success was on average 0.83 ± 0.38 ($n=35$). When hatched, fledging probability of second clutches differed per manipulation treatment ($\chi^2_2 = 8.53$, $p<0.02$). Fledging probability of second clutches was reduced in nests of parents of the 'enlarged' category in 2000 and 2002 (figure 1). No systematic trend occurred between the experimental treatments during first clutches and laying date of second clutches that could explain this pattern. Only in 2002 the offspring of second clutches did recruit in the subsequent breeding season (table 1).

(c) *Local survival of parents*

Local survival of the parents differed between years ($\chi^2_2 = 6.71$, $p<0.04$; table 1). More individuals manipulated in 2003 bred a year later than those manipulated in the years 2000 and 2002. The data suggested a negative effect of clutch enlargement on local survival in 2 out of 3 years (2000 and 2002; figure 2), but the interaction between year and manipulation was non-significant ($\chi^2_4 = 2.92$, $p=0.57$). In the two years, however, clutch enlargement did significantly affect local survival of the parents to the subsequent breeding season ($\chi^2_2 = 6.28$, $p=0.04$; no year effect, $\chi^2_1 = 0.03$, $p=0.98$; see §4). Survival probabilities of males and females were not found to differ from each other in either analyses (sex effect in 3 years: $\chi^2_1 = 0.39$, $p=0.53$, controlled for year, manipulation and their interaction; sex effect in 2 years: $\chi^2_1 = 1.65$, $p=0.20$, controlled for treatment).

(d) *Fecundity of females in the following year*

In the breeding season following the year of manipulation, clutch size differed both with treatment categories and year (treatment effect controlled for year, $\chi^2_2 = 7.5$, $p=0.02$; year effect controlled for treatment, $\chi^2_2 = 13.9$, $p<0.001$). Birds subjected to clutch reduction produced smaller clutches (table 1). Comparisons of laying dates between the year of manipulation and the subsequent year revealed no difference between treatment categories ($\chi^2_2 = 1.7$, $p=0.42$, controlled for year), but did between experimental years ($\chi^2_2 = 45.9$, $p<0.001$).

4. DISCUSSION

By manipulating the clutch size during the incubation phase only, we were able to quantify the effect of clutch size on the fitness costs of incubation. The experimental treatment revealed no detectable fitness costs in the short term, but in the long term it did. During the two years (2000 and 2002) with second clutches, offspring of second clutches had lower fledging probability when parents incubated enlarged first clutches. In the same two years, local survival probability of parents incubating enlarged first clutches was reduced.

We used the clutch size manipulations as a tool to measure the fitness costs in relation to alternative options

Table 1. Overview of the fitness components (mean \pm s.d.) per year and treatment category for the nests used in the analyses.

	2000			2002			2003		
	reduced	control	enlarged	reduced	control	enlarged	reduced	control	enlarged
first clutch (<i>n</i>)	11	11	13	13	18	15	11	13	14
clutch size	9.5 \pm 0.7	9.0 \pm 0.6	9.4 \pm 1.2	9.5 \pm 1.1	9.2 \pm 1.0	9.3 \pm 1.1	8.1 \pm 0.9	8.0 \pm 1.2	8.1 \pm 1.4
hatching probability	0.91 \pm 0.12	0.95 \pm 0.10	0.88 \pm 0.18	0.88 \pm 0.12	0.92 \pm 0.09	0.89 \pm 0.12	0.90 \pm 0.07	0.94 \pm 0.07	0.95 \pm 0.07
probability of success	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	0.73 \pm 0.5	1 \pm 0.0	0.93 \pm 0.3
fledging probability	0.93 \pm 0.16	0.94 \pm 0.10	0.88 \pm 0.15	0.92 \pm 0.14	0.90 \pm 0.14	0.94 \pm 0.11	0.83 \pm 0.20	0.87 \pm 0.14	0.91 \pm 0.19
local recruitment probability	0.065 \pm 0.08	0.054 \pm 0.06	0.046 \pm 0.09	0.145 \pm 0.10	0.141 \pm 0.18	0.073 \pm 0.10	0.094 \pm 0.12	0.136 \pm 0.13	0.120 \pm 0.11
second clutch (<i>n</i>)	4	4	6	7	7	7	1	0	0
probability of second clutch	0.36 \pm 0.50	0.36 \pm 0.50	0.46 \pm 0.52	0.54 \pm 0.52	0.39 \pm 0.50	0.47 \pm 0.52	0.03 \pm 0.30	0	0
clutch size	7.3 \pm 0.5	7.3 \pm 0.5	7.2 \pm 1.0	6.9 \pm 0.9	7.7 \pm 1.4	8.1 \pm 0.9	6 \pm 0.0	—	—
hatching probability	0.68 \pm 0.21	0.65 \pm 0.38	0.80 \pm 0.25	0.98 \pm 0.05	0.83 \pm 0.20	0.95 \pm 0.06	1 \pm 0.0	—	—
probability of success	1 \pm 0.0	0.5 \pm 0.58	0.5 \pm 0.55	0.86 \pm 0.38	1 \pm 0.0	1 \pm 0.0	1 \pm 0.0	—	—
fledging probability	1 \pm 0.0	1 \pm 0.0	0.77 \pm 0.13	0.86 \pm 0.12	0.87 \pm 0.18	0.74 \pm 0.19	0.83 \pm 0.0	—	—
local recruitment probability	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0	0.065 \pm 0.09	0.10 \pm 0.14	0 \pm 0.0	—	—
local survival parents	0.36 \pm 0.45	0.45 \pm 0.27	0.27 \pm 0.33	0.38 \pm 0.36	0.47 \pm 0.40	0.23 \pm 0.26	0.50 \pm 0.39	0.54 \pm 0.38	0.57 \pm 0.39
female	0.36 \pm 0.50	0.45 \pm 0.52	0.38 \pm 0.51	0.38 \pm 0.51	0.56 \pm 0.51	0.27 \pm 0.46	0.55 \pm 0.52	0.38 \pm 0.51	0.57 \pm 0.51
male	0.36 \pm 0.50	0.45 \pm 0.52	0.15 \pm 0.38	0.38 \pm 0.51	0.39 \pm 0.50	0.20 \pm 0.41	0.45 \pm 0.52	0.69 \pm 0.48	0.57 \pm 0.51
subsequent season ^a (<i>n</i>)	4	5	5	5	10	4	6	5	8
clutch size	8.8 \pm 1.0	9.4 \pm 0.9	8.6 \pm 0.9	7.4 \pm 1.7	7.9 \pm 1.0	8.0 \pm 0.8	7.0 \pm 2.0	9.2 \pm 1.3	9.8 \pm 1.8
laying date	30.0 \pm 0.8	29.0 \pm 2.8	32.0 \pm 2.0	25.8 \pm 2.3	24.2 \pm 7.3	19.3 \pm 6.2	21.5 \pm 5.4	12.4 \pm 5.7	14.9 \pm 9.5

^a For females only.

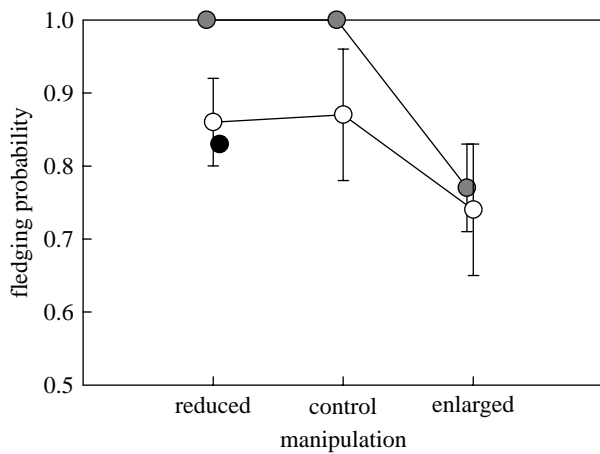


Figure 1. Fledging probability of offspring from second clutches per manipulation category for 3 years (grey circles, 2000; white circles, 2002; black circles, 2003).

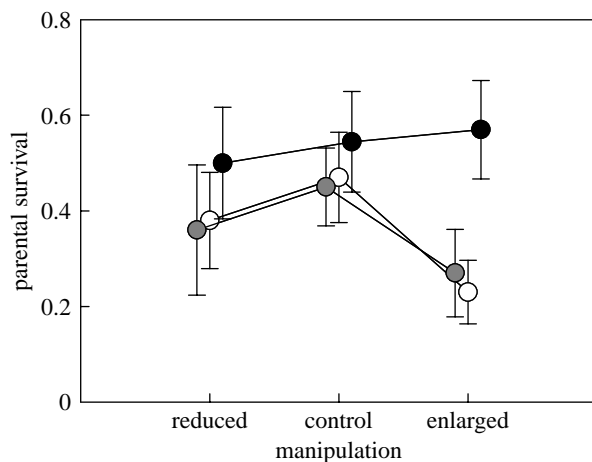


Figure 2. Average local survival of parents per manipulation category for 3 years (grey circles, 2000; white circles, 2002; black circles, 2003).

in reproduction. With this approach, we assumed parents to respond to the experimental treatment as if it were a result of their own decision (Lessells 1993). In our study, this assumption may have been violated, as parents of whom we reduced clutch size were more likely to desert their nest. The decision to desert the nest may be related to parental quality (Verboven & Tinbergen 2002). One possible indicator of parental quality is clutch size. We found no significant correlation between the probability of nest desertion and the original clutch size ($\chi^2_1 = 2.8$, $p = 0.09$), which suggests that there was no quality difference between parents of the reduced category that stayed and those that left. Because other quality differences may have affected the probability of desertion, we repeated our analyses by excluding the reduced categories. These analyses revealed no substantial different results.

The clutch size manipulations revealed no fitness costs of incubation in the short term, judged from the fact that offspring fitness of first clutches did not differ between the treatment categories. These results suggest that parents incubating enlarged clutches did care equally as well for their young during the nestling phase as those incubating either reduced or control clutches. However, when these parents had to care for offspring of second clutches, the fledging probability of these offspring was reduced.

Similarly, Reid *et al.* (2000b) found effects in second clutches after parents were faced with increased investment during the incubation phase of first clutches. Parents are apparently not willing to jeopardize offspring fitness of the first clutch, and postpone paying the costs to the second clutch. Alternatively, parents may have reduced their investment in immune function when rearing first clutches, which may increase the chance of infections and possibly reduce their condition when rearing second clutches (Siikamäki *et al.* 1997; Hanssen *et al.* 2005). Depending on whether parents can postpone these costs, the outcome of the trade-off between investment in current and future breeding attempts may be different. This may explain why in addition to studies that reported fitness costs for offspring in enlarged clutches (Moreno *et al.* 1991; Siikamäki 1995; Reid *et al.* 2000b; Engstrand & Bryant 2002), at least as many studies found no such costs (e.g. Smith 1989; Sanz 1997; Cichon 2000; Visser & Lessells 2001; appendix A).

The differences between the years could be the result of the change in manipulation intensity between 2002 and 2003. In 2002, clutch enlargement was performed with either two or three eggs. Although data are limited, there is no indication that local survival of parents receiving two or three additional eggs differed in the expected direction from each other (0.25 and 0.22, respectively; $\chi^2_1 = 0.03$, $p = 0.86$; for comparison, local survival of parents with control clutches = 0.47). We favour the explanation that ecological circumstances have differed between the years. The high occurrence of second clutches together with the fact that birds laid early and had larger clutches in 2000 and 2002 as compared to 2003 may indicate annual differences in selection pressures on clutch size (Verhulst 1998; Tinbergen & Sanz 2004). In the study of Visser & Lessells (2001), who also measured the costs of incubation in great tits, a similar correlation as in our study exists; in the one year with second clutches, the local survival of parents (here estimated as the differences in mean local parental survival of both males and females between their free eggs and free chicks treatments) was lower than that in the other year without second clutches (0.14 versus 0.06 parental units). Thus, although we lacked statistical power to show the difference between the responsive and the non-responsive years, the data strongly suggest that clutch size-related costs of incubation exist in some years.

It is unlikely that the reduced local survival of parents in the enlarged category was caused by differential dispersal. After first settlement, parent great tits disperse only over short distance (less than 160 m) between two breeding attempts (Tinbergen 2005). Therefore, the reduced local survival in this category is likely the direct result of reduced investment in self-maintenance. Studies that investigated the physiological consequences of incubating enlarged clutches report reduced condition (Hanssen *et al.* 2005) or immune competence (Siikamäki *et al.* 1997; Hanssen *et al.* 2005).

In the breeding systems with uniparental incubation, males and females have clearly different tasks during the incubation phase. It is therefore surprising that the manipulation effect on local survival did not differ between males and females. For the female, the potential cost of reproduction is apparent: she incubates the eggs during three-quarters of the daylight period and therefore needs to trade-off investment in the offspring with

self-maintenance. The male, however, does not incubate and the constraining effect of a large clutch on the survival via his role during the incubation phase is less clear. Males have been observed to provision their mate during incubation, either inside or outside the nest-box (Royama 1966). In reaction to the clutch enlargement, males may increase their provisioning rate to their partner (Sanz 1997), and thereby reduce their survival in favour of current reproduction. Also, males may take their females to good foraging sites and the effort of males to patrol their territory and keep track on the good foraging sites may increase with the females' needs when they are incubating enlarged clutches. An alternative and perhaps more plausible explanation is that the experimentally induced costs during incubation may negatively affect the female's provisioning effort during the nestling phase and males may compensate for this by working harder (Sanz *et al.* 2000), thereby reducing their probability of breeding also in the next season.

To quantify the importance of the costs of incubation for the selection of clutch size on the basis of the quantitative effects, we estimated the overall fitness of rearing manipulated clutches from incubation onwards (figure 3). To do this, we integrated the results of the current study with that of brood size manipulations from the earlier study on the same population by Tinbergen & Sanz (2004). The average number of first clutch recruits per nest per manipulation category in relation to manipulated clutch size was estimated using the recruitment probability from the brood manipulation study only. This was done because we did not find an effect of clutch size manipulation on offspring fitness during incubation. The number of recruits from second clutches was estimated from the clutch size manipulation during incubation study (this study). For the parental fitness component (the annual average parental survival per manipulation category, table 1), we used the effect of the clutch size manipulation during incubation only, because there were no indications of a survival effect of brood size manipulation. Thus, we assume fitness effects during incubation and rearing offspring to be additive and also assume no effects of egg production (but see Heaney & Monaghan 1995; Visser & Lessells 2001; Kalmbach *et al.* 2004). The result shows that the costs of incubation were strong enough to change the positive selection on clutch size during the nestling phase into stabilizing selection in the years 2000 and 2002 (figure 3). In the third year (2003), selection remained directional and positive. One explanation for this pattern is that fitness has a constant and quadratic relation to clutch size over the years, as suggested by the solid line in figure 3. In this view, it would depend on the average clutch size of the year in which direction selection would act.

We believe that the effects we found are not specific for our population. The work of Visser & Lessells (2001) suggests a survival cost for incubation of extra eggs and found an effect on fecundity in the next year Hanssen *et al.* (2005). Whether such a clutch size-related fitness cost of incubation will affect the optimal clutch size depends on the exact shape of the parental fitness curve with clutch size. In our case, positive selection during the nestling phase changed to stabilizing selection. In populations where the selection as measured during the nestling phase was stabilizing, no change in optimal clutch size would be

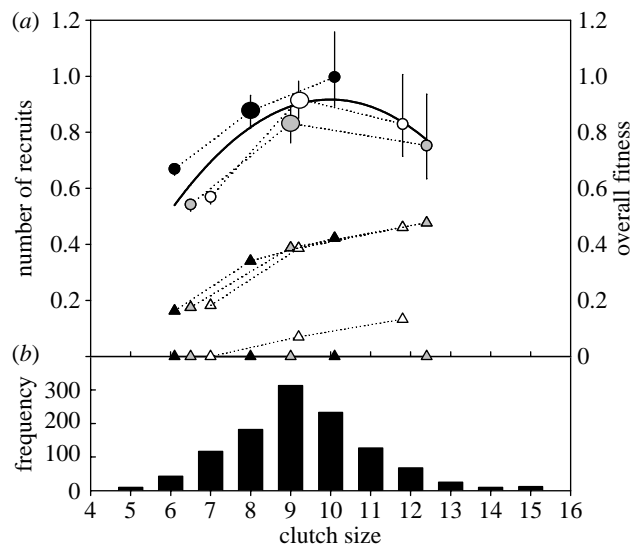


Figure 3. (a) Fitness estimates in relation to manipulated clutch size from incubation onwards (circles; large symbols are controls) for 3 years (grey circles, 2000; white circles, 2002; black circles, 2003). The lower group of points indicate the second clutch fitness component, the middle group of points indicate the first clutch fitness component while the top group of points indicate overall fitness (the line is the second order regression through these points). Overall fitness was calculated as the average survival of the parents, the first clutch recruits divided by two ($R_{\text{first clutch}}$) and the second clutch recruits divided by two ($R_{\text{second clutch}}$) following Tinbergen & Sanz (2004). Survival probabilities for the parents and the number of female recruits from second clutches were derived from the clutch size manipulations (this study) and the number of female recruits of the first clutch from the brood size manipulations (Tinbergen & Sanz 2004). Estimates were derived as follows: $R_{\text{first clutch}} = \text{CS} \times \text{HP} \times \text{NS} \times \text{FP} \times \text{RP}$, where CS is the clutch size (taken from this study); HP, hatching probability (this study); NS, probability that a nest is successful (taken from the brood manipulation study); FP, fledging probability; and RP, recruitment probability (both taken from the brood manipulation study). Mean recruitment of the second clutch was estimated as $\text{MR}_{\text{second clutch}} = q \times R_{\text{second clutch}}$, where q is the probability of a second clutch and $R_{\text{second clutch}}$ represents the number of female recruits from a successful second clutch (both taken from this study). The indicated ranges in the overall fitness represent the variation between years in the brood size study and were estimated by using mean values for the two most extreme years. (b) Frequency distribution of observed clutch sizes in the Lauwersmeer population for the years 1994–2003.

expected, unless the cost of incubation would change not only for the enlarged clutches but also for the whole range of clutch sizes.

In contrast to the earlier conclusions of Tinbergen & Sanz (2004), our experiments show that the observed clutch size in our study population may maximize fitness when we account for the clutch size-related fitness costs of incubation. Because the temporal variation in selection pressure on clutch size is considerable and selection acts differently in the different reproductive phases, there is still a lot of scope for work in this field. Not only experiments isolating the effects within the different phases are needed, but also experiments studying the interaction between the phases are needed to determine the costs and benefits related to clutch size.

Table 2. Overview of studies that manipulated clutch size during the incubation phase to measure the fitness consequences in both (A) altricial and (B) precocial birds; results of clutch enlargement (0, no effect; —, negative effect). (1, Species; 2, observed clutch size; 3, clutch size used in the experiment; 4, number of eggs manipulated; 5, treatment category (R, reduced; C₁, control; no eggs were transported or exchanges; C₂, control; part of the clutch was transported or exchanges between nests; C₃, both control categories (C₁ + C₂) were used; E, enlarged); 6, experimental approach (I, only the costs of incubation are studied (clutch sizes were manipulated during incubation and restored at hatching); J, costs of both incubation and rearing chicks are studied (clutch sizes were manipulated during incubation and not restored at hatching); K, costs of incubation are studied (the performance of birds receiving extra eggs from the incubation phase onwards is compared with those receiving extra chicks from the nestling phase onwards)); 7, triplets/pairs were matched for date in the field (y, yes; n, no); 8, triplets/pairs were matched for clutch size in the field (y, yes; n, no); 9, (indication of) sample size per manipulation category; 10, number of replicates (years). For offspring of the manipulated clutches: 11, hatching probability; 12, fledging probability; 13, recruitment probability. For parents of the manipulated clutches: 14, probability of second clutch; 15, survival; 16, laying date in the subsequent breeding season; 17, clutch size in the subsequent breeding season; 18, References: 1 (Engstrand & Bryant 2002), 2 (Smith 1989), 3 (Cichon 2000), 4 (Moreno *et al.* 1991), 5 (Visser & Lessells 2001), 6 (Ilmonen *et al.* 2002), 7 (Siikamäki 1995), 8 (Sanz 1997), 9 (Moreno & Carlson 1989), 10 (Reid *et al.* 2000b), 11 (Heaney & Monaghan 1995), 12 (Heaney & Monaghan 1996), 13 (Hanssen *et al.* 2003), 14 (Hanssen *et al.* 2005), 15 (Larsen *et al.* 2003) and 16 (Wallander & Andersson 2002).)

A																	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
barn swallow <i>Hirundo rustica</i>	2–7	4–5 [?]	2	R,C ₃ ,E	I	y	?	42,53,44	2	—							1
blue tit <i>Parus caeruleus</i>	≥9	≥9	≥3	R,E	J	y	y	10,10	1	0							2
collared flycatcher <i>Ficedula albicollis</i>	4–8	6–7	2	R,E	I	y	y	26 ^a	1	0	0						3
collared flycatcher <i>F. albicollis</i>	?	?	2	R,C ₂ ,E	J	y	y	19,10,16	1	—							4
great tit <i>Parus major</i>	?	?	2	C ₁ ,E	K	n	n	41,26	2	0	0	0	0	—/0	—	0	5
pied flycatcher <i>Ficedula hypoleuca</i>	3–9	6–7	2	R,C ₂ ,E	I	y	y	18,11,13	1	0	—						6
pied flycatcher <i>F. hypoleuca</i>	3–8	?	2	R,C ₃ ,E	J	y	n	53,99,53	3	—							7
pied flycatcher <i>F. hypoleuca</i>	4–7	5–6	2	R,C ₁ ,E	J	y	y	21,33,20	1	0	—			0	0	0	8
pied flycatcher <i>F. hypoleuca</i>	6–8	6–8	2	R,C ₂ ,E	J	y	y	17,25,15	1	0							9
starling <i>Sturnus vulgaris</i>	4–7	4–5	1 + 1 ^b	C ₂ ,E	I	y	n	17,17	1	—	0						10
B																	
common tern <i>Sterna hirundo</i>	1–3	2	1	C ₁ ,E	K	y	y	17,18	1	0	—						11
common tern <i>S. hirundo</i>	1–3	2	1	C ₁ ,E	I	y	y	20,21	1	0	0						12
eider <i>Somateria mollissima</i>	3–6	4–5	1	R,C ₃ ,E	J	n	n	31,49,31	3	— ^c				0			13
eider <i>S. mollissima</i>	3–6	3–6	1,2,3 ^d	R,E	J	n	n	24,30	2					0	—	—	14
northern lapwing <i>Vanellus vanellus</i>	4	4	1	R,C ₁ ,E	J	y	y	15,18,18	1	—							15
redshank <i>Tringa totanus</i>	4	4	1	C ₂ ,E	J	n	y	11,15	1	0							16

^a Overall sample size is given, as sample size could not be separated for the categories.

^b One of the two eggs added to the clutch was a model egg with thermistor.

^c Results are given for birds with four egg clutches.

^d Birds received a fixed number of eggs during incubation; either three (low quality) or six (high quality).

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APPENDIX A

Table 2.

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