Behav Ecol Sociobiol (2006) 60: 184–194
DOI 10.1007/s00265-005-0155-z

ORIGINAL ARTICLE

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Brood size manipulation affects frequency of second clutches in the blue tit

Received: 10 May 2005 / Revised: 15 December 2005 / Accepted: 18 December 2005 / Published online: 7 February 2006

Abstract The reproductive trade-off hypothesis predicts that the investment made in current reproduction determines the breeders' future fitness as a consequence of intra-or inter-generational reproductive costs. Long-lived species are expected to favour their own reproductive value at the expense of their offspring, hence incurring in inter-generational costs, whereas short-lived species are expected to invest in the current breeding attempt even at the expense of their own survival, thus incurring in intra-generational costs. We tested whether intensity of current reproductive effort has intra-or inter-generational costs in a short-lived bird, the blue tit *Parus caeruleus*, with a brood size manipulation experiment. We expected more intra-generational (parental reproduction and/or survival) than inter-generational (offspring quality and survival) reproductive costs. We found that parental effort, measured as the hourly rate of parental visits to nests, increased gradually with experimental manipulation. Brood size manipulation resulted in a gradual increase in the number of fledglings per nest from reduced to increased treatments. We found an effect of the manipulation on the probability of making a second clutch, with adults rearing enlarged broods being less likely to undertake such a second reproduction during the season compared to those rearing control or decreased broods. We found no evidence of other reproductive costs; neither as adult weight after manipulation, apparent parental local survival, apparent offspring local survival or local recruitment. Although the results seem to support the a priori expectations, alternative explanations are discussed.

Keywords *Parus caeruleus* · Brood size manipulation · Parental effort · Reproductive costs · Non-breeding birds

Introduction

Resource allocation for each reproductive episode over the lifetime is one of the main paradigms of life-history evolutionary theory (Roff 1992; Stearns 1992). Whenever reproductive costs exist, parents face a compromise between current and future reproductions and survival in order to maximise fitness (Willians 1966; Roff 1992; Stearns 1992). One of the crucial periods influencing this balance is the rearing period (Drent and Daan 1980) when breeders must decide how to allocate their resources between themselves and their offspring, with implication on their future fitness. The resolution of this trade-off may depend on life history strategies. For instance, long- lived species are expected to favour their own reproductive value at the expense of their offspring (Drent and Daan 1980), hence incurring in inter-generational costs (paid by the offspring). On the contrary, short-lived species are expected to invest in the current breeding attempt even at the expense of their own survival (Stearns 1992), thus mainly incurring in intra-generational costs (paid by the parents), although offspring of short-lived species might also pay the costs under food shortage conditions.

Costs incurred by parents having a certain brood size may be studied by experimental manipulations of brood size (Lessells 1991; Stearns 1992). Such imposed costs may be detectable as intra- or inter-generational costs. Surprisingly, experimental evidence often does not support the expectations in relation to the kind of life history strategies. For instance, the existence of a positive relationship between parental effort, and inter-generational costs has been shown both for short- and long-lived birds in terms

Communicated by M. Soler

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of offspring recruitment rate, quality and future fitness (Table 1). However, in other populations of the same species, or in other species, this relationship was either negative or inexistent (Table 1). Furthermore, intra-generational costs of reproduction have also been demonstrated for both short- and long-lived birds in terms of reduced probability of laying a second clutch, increase in the interval between the first and the second brood, adult weight loss during the nestling period, and parental survival or depression of the immune system (Table 1). Other studies have failed to find any such costs (Table 1).

There are some reasons for this variability. In particular, some intra-generational costs of reproduction may not be easily studied and compared among populations. Environmental quality may affect parental effort differentially (Tammaru and Hõrak 1999). For instance, in double-brooded species, second clutches do not occur at high latitudes, thus other reproductive costs are expected there. In addition, the magnitude of the experimental manipulation could affect reproductive costs directly or in interaction with environmental quality (Tammaru and Hõrak 1999).

The aim of this study is to test whether the intensity of current reproductive effort has intra- or inter-generational costs in a short-lived bird, the blue tit, in a temperate region. Blue tits are small hole-nesting passerines. In our study area, some individuals undertake a second clutch every year. Other studies have focused on costs of reproduction in this species (see Table 1). In our study, we used experimental brood size manipulations to modify parental effort along a gradient. We investigated both intra-generational (occurrence of second clutches, parental weight after manipulation and parental apparent survival) and inter-generational costs (offspring quality and probability of producing locally recruiting young) caused by the intensity of current reproduction, expecting intra-generational more than inter-generational costs. Our goal was to assess many potential costs for the same individuals simultaneously, as the sensitivity to the imposed costs may differ among phenotypes as well as life history parameters. Furthermore, some external factors may affect the sensitivity to our manipulations differentially. For instance, the negative effects of increased reproductive effort on adult survival or offspring quality may occur preferentially under stressful circumstances (Bell and Koufopanou 1986). Our main prediction was that blue tits should show more intra-generational than inter-generational costs because they are short-lived birds.

Materials and methods

Study system

The study was conducted in 2002 and 2003 in a 500 ha mixed deciduous forest of the Parc Régional de la Forêt d'Orient, in central France. The main tree species are oaks (*Quercus petraea*) and hornbeams (*Carpinus betulus*). About 1,000 nest boxes were scattered over the forest in 1999. Blue and great tits (*Parus major*) commonly use such nest boxes.

The blue tit is an 11 g, hole nesting passerine, common in the woodlands in Europe (Cramp and Simmons 1988). It usually breeds monogamously and although only the female incubates the eggs and broods the young, both sexes feed and clean the young. The nestling period is approximately 19 days. In the study area it is a resident bird, and a small proportion of the population makes a second breeding attempt after successfully raising a first brood [the percentage of second clutches in 2002 was 7.08% (n=8) for broods of unchanged size]. Occurrence of second clutches is not limited by nest-box availability because the mean occupation rate in the forest was 44.91% for both years (including other bird species and small mammals) during the first clutch and less than 20% at the time of the second clutch.

Each year, nest boxes were visited regularly from nest building (early April) to fledging to determine laying dates, clutch sizes, incubation and hatching dates and fledging success. Adults and chicks were captured in the nest box when chicks were between 8 and 13 days old. Adults were ringed with aluminium and plastic colour rings, whilst only aluminium rings were used for chicks. Subsequent movements of adults were recorded later in the 2002 season and during the 2003 season. In 2002, we captured 335 adult and 2,594 juvenile blue tits through all the breeding season, corresponding to 57.6% of the breeders and 81.1% of the juveniles. In 2003, we captured 206 adult blue tits, which correspond to 76.9% of the breeders. In spite of these high figures, we only obtained 24 locally recaptured adult birds. At ringing we measured: (1) body mass with a Pesola spring balance with a precision of 0.1 g and (2) wing and tarsus length with a sliding calliper to the nearest 0.1 mm. Adults were sexed according to the presence or absence of a brood patch. In most cases, wing plumage characteristics allowed us to distinguish between yearlings and older breeders.

The probability of having a second brood was determined by catching the parents during the second nesting phase. Annual apparent local survival was estimated on the basis of recaptures of birds during the following breeding season in the study area (see above for recapture rates). The probability of producing locally breeding offspring by a pair in a single breeding event is termed local recruitment rate. The last two estimates, which we term "apparent local survival" and "local recruitment rate", are in fact the product of four differential parameters: survival rate, return rates to the study area, breeding probability, as well as the probability to be recaptured.

Brood size manipulations

Brood size manipulations were carried out in 2002 at two nested scales: the scale of a patch and that of a nest. We later call the treatment at the scale of the patch as "Patch-Treatment", and that at the scale of the nest as "Nest-Manipulation". Although in this paper we concentrate on

Table 1 Review of the results of experimental clutch or brood size manipulations as a way to modify the reproductive effort to study intra- or inter-generational reproductive costs in birds

Intra-generational costs				
Cost	Species	Effect	Sex	Reference
Short-lived birds				
Second broods probability	*Parus major*	−	F + M	Tinbergen (1987), Tinbergen and Both (1999)
	Parus major	0	F + M	Sanz and Tinbergen (1999)
	Motacilla cinerea	0	F + M	Klemp (2000)
Time between two clutches in the season	*Parus major*	−	F + M	Sanz and Tinbergen (1999)
	Motacilla cinerea	0	F + M	Klemp (2000)
Duration of post-fledging care	*Parus major*	0	F + M	Verhulst and Hut (1996)
Parental inmune system	*Ficedula hypoleuca*	−	F	Moreno et al. (1999)
	Parus major	−	F + M	Hõrak et al. (1998)
Adult weight during the nesting phase	*Ficedula hypoleuca*	−, 0	F, M	Moreno et al. (1995)
	Parus major	−	F + M	Tinbergen and Verhulst (2000)
	Parus major	0	F + M	Sanz and Tinbergen (1999)
	Parus caeruleus	−	F + M	Nur (1984a), Merilä and Wiggings (1997)
	Parus caeruleus	−	F	Fargallo and Merino (1999)
	Parus montanus	0	F + M	Orell et al. (1996)
	Iridoprocne bicolour	0	F + M	De Steven (1980)
Time of moult	*Ficedula hypoleuca*	0	F + M	Sanz (1997)
Parental blood infection	*Parus caeruleus*	−	F	Fargallo and Merino (1999), Merilä and Andersson (1999), Stjernman et al. (2004)
Parental survival	*Hirundo rustica*	−		Saino et al. (1999)
	Iridoprocne bicolour	0	F	De Steven (1980)
	Ficedula hypoleuca	−	M	Askenmo (1979)
	Ficedula albicollis	0	F + M	Gustaffson and Sutherland (1988)
	Parus major	−	F + M	Kluyver (1951), Lindén (1988)
	Parus major	+, 0	F, M	Hõrak (2003)
	Parus major	0	F + M	Boyce and Perrins (1987), Pettifor et al. (1988), Tinbergen (1987), Tinbergen and Both (1999)
	Parus caeruleus	−, 0	F, M	Nur (1984a)
	Parus caeruleus	0	F + M	Pettifor (1993b)
	Parus montanus	0	F + M	Orell and Koivula (1988)
	Corvus frugilegus	0	F + M	Røskaft (1985)
	Passer domesticus	0	F + M	Hegner and Wingfield (1987)
Parental survival and subsequent breeding success	*Ficedula hypoleuca*	0	F	Sanz (1997)
Parental future fecundity	*Parus caeruleus*	−	F + M	Nur (1988)
Long-lived birds				
Second broods probability	*Tyto alba*	0	F + M	Roulin et al. (1999)

Table 1 (continued)

Cost	Species	Effect	Sex	Reference
Intra-generational costs				
Adult weight during the nesting phase	*Puffinus puffinus*	0	F + M	Harris (1966)
	Sula capensis	0	F + M	Jarvis (1974)
	Sula nebouxii	−, 0	F, M	Velando and Alonso (2003)
	Falco tinnunculus	−, 0	F, M	Dijkstra et al. (1990)
	Rissa tridactyla	−, 0	F, M	Jacobsen et al. (1995)
	Larus glaucescens	−	F + M	Reid (1987)
	Larus furcatus	0	F + M	Harris (1970)
	Aegolius funereus	0	F + M	Korpimäki (1988)
Parental survival	*Falco tinnunculus*	−	M	Daan et al. (1996), Dijkstra et al. (1990)
	Larus glaucecens	−	F + M	Reid (1987)
	Rissa tridactyla	−		Jacobsen et al. (1995)
	Creagrus furcatus	0	F + M	Harris (1970)
	Aegolius funereus	0	F + M	Korpimäki (1988)
Inter-generational costs				
Short-lived birds				
Local recruitment rate	*Parus major*	+		Hõrak (2003), Perrins and Moss (1975), Pettifor et al. (1988), Tinbergen and Daan (1990)
	Parus major	0		Tinbergen and Both (1999)
	Parus major	−		Verhulst (1995)
Offspring survival after 3 months	Parus caeruleus	+		Pettifor (1993a)
Offspring quality	*Parus major*	−		Rytkönen and Orell (2001)
Offspring future fitness	*Parus caeruleus*	−		Blondel et al. (1998)
Long-lived birds				
Offspring quality	*Tyto alba*	−		Roulin et al. (1999)

[a] Positive, negative and non-significant effects of the experimental manipulation are indicated by +, − and 0, respectively. When possible, we show two different effects representing females on the left and males on the right

M male parent; *F* female parent

the effect of Nest-Manipulations we controlled for the effect of Patch-Treatment. Breeding patches were randomly assigned to one of the four following Treatments (10 replicates of each): decreased (D, *n*=32 nests), non-manipulated control (no manipulation at all; C1, *n*=56 nests), manipulated control (leaving brood size unchanged by swapping one or two nestlings between two nests of those patches; C2, *n*=14 nests), or increased (I, *n*=35 nests). In each manipulated patch, we only performed manipulation in a portion of the nests. One or two nestlings younger than two-day old were moved from D to I patches between nests matched by hatching dates. Nestlings were also moved between C2 nests. Each patch had approximately the same dimensions and they included a mean number of 25 nest boxes.

Thus, we created brood sizes deviating from their original sizes by −2, −1, 0, +1 and +2 nestlings (Nest-Manipulation values). All plots contained non-manipulated nests. Experimental manipulations were carried out between the first hatching date (+ 2 days) and the end of the hatching peak. Differences in sample size in complementary experimental categories were due to predation. We excluded from the analyses three nests that were known to have polygynic males. Neither laying dates (one-way ANOVA: Nest-Manipulation effect, $F_{5,131}$=0.46, *P*=0.81) nor natural clutch size (one-way ANOVA: Nest-Manipulation effect, $F_{5,131}$=0.51, *P*=0.77) differed among treatments. Furthermore, none of the morphological measurements differed among treatments (Wing length, one-ANOVA: Nest-Manipulation effect, $F_{5,105}$=0.75, *P*=0.59 and $F_{5,111}$=0.15, *P*=0.98 for males and females, respectively; Tarsus length, one-ANOVA: Nest-Manipulation effect, $F_{5,105}$=0.57, *P*=0.72 and $F_{5,111}$=0.52, *P*=0.76 for males and females, respectively). Female age did not differ among treatments (one-way ANOVA: Nest-Manipulation effect, $F_{5,74}$=3.18, P=0.67) either, which is an important result because female age is an important variable likely to affect reproductive parameters.

Behavioural observations

During the nestling period of 2002, we observed nest boxes from a distance (about 50 m) in order to estimate parental effort. We recorded the number of parental visits to the nest during random 30-min periods. Because the time when provisioning is maximal occurs in the late nestling period (Deerenberg et al. 1995; Moreno et al. 1995; Verhulst and Tinbergen 1997), we started observations at day 9 of the nestling period at the earliest. We recorded any kind of parental visits in order to account for every aspect of parental effort, which includes food provisioning as well as brooding or cleaning (Sanz and Tinbergen 1999).

Nest observations involved nests of the different Nest-Manipulation treatments. When more than one observation per nest was available, we only considered the first observation after ringing to avoid pseudo-replication. The age at which broods were observed did not differ among Nest-Manipulation categories (one-way ANOVA: $F_{5,51}$=0.68, P=0.64). From each observation we calculated the number of parental visits per hour, number of parental visits per hour and young and the total amount of time spent by the parents in the nest per hour. Period of day (day hours from 8 to 20 h grouped by 4 h classes) did not affect the rate of parental visits to the nest (one-way ANOVA: $F_{2,52}$=0.65, P=0.52), the rate of parental visits per chick (one-way ANOVA: $F_{2,52}$=1.87, P=0.16) or the rate of time spent by parents on the nest (one-way ANOVA: $F_{2,52}$=2.28, P= 0.11). Thus, we used all the observations in analyses irrespectively of the hour of sampling. As identification of parents was not always possible due to their fast entrance in nests, we calculated all variables for both parents together.

Statistical analyses

Each nesting attempt was used as a statistically independent observation in the analyses of effects of Nest-Manipulation on chick number and condition per nest, parental investment, and local recruitment rate. We performed ANOVA or analysis of covariance (ANCOVA) models with the GLM procedure of SAS [SAS 1999] for continuous dependent variables and logistic regression models with the GENMOD procedure of SAS for binary dependent variables. However, in the analyses on the probability of having a second brood and of being a local breeder the following season, we introduced the nest box nested in the patch and the patch as random effects to account for the fact that adults and chicks from the same nest and patch were not independent. Then, analyses were performed for individuals (generalized linear mixed models (GLMMs), using SAS Macro program GLIMMIX (Littell et al. 1996) for these binary dependent variables). The significance of square of manipulation terms was also tested in all models. When analysing the effect of the experiment on the probability of having a second brood, we also introduced in models the laying date of first clutches as a co-variate to account for its effect.

Habitat copying has been shown to be a widespread strategy in animal breeding habitat selection (please see review in Danchin et al. 2001, 2004). Therefore, the Patch-Treatment performed in breeding plots is likely to influence both intra- (dispersal between first and second clutch) and inter-seasonal dispersal decisions (dispersal between two consecutive reproductive seasons). Because dispersal is likely to be one of the parameters determining some of our study variables, Patch-Treatment was thus tested as a fixed continuous explanatory variable to control for its effect in analyses on the probability of making a second clutch and apparent local survival. Because Nest-Manipulation and Patch-Treatment were highly correlated (r=0.889, P< 0.0001) we could not input them simultaneously in the statistical models. We thus test them separately. In all analyses, the Nest-Manipulation was introduced as a continuous effect to account for its gradual intensity. The experimental Nest-Manipulation performed included two control groups (non-manipulated control, C1 and manipulated control, C2). Thus, to account for the possible effect of the actual Nest-Manipulation, before every analysis we tested for differences between these two controls and later grouped them as they never showed any statistical differences.

Finally, we introduced sex in analyses of adult apparent local survival because this variable is known to influence dispersal decisions (Greenwood 1980). For each analysis, we specify the starting model. Model selection was carried out by removing, one by one, the effects that were furthest from statistical significance, starting with the highest-order interactions down to the main effects. We used body mass as chick condition in conjunction with wing length and chick age as co-variables in the model.

Results

Effects of nest-manipulation on chick number and condition

There were no differences between the two control groups (nests from C1 and C2 breeding plots) either in the number of fledglings per nest (one-way ANOVA: Nest-Manipulation effect, $F_{1,68}$=0.01, P=0.92) or in their body condition (ANCOVA: Nest-Manipulation effect, $F_{1,63}$=0.07, P= 0.80). Chick body condition was only affected by the interaction between chicks' wing length and age (ANCOVA: interaction term wing length *age, $F_{1,64}$=13.06, P< 0.001). No other interaction or main effect affected body condition (in ANCOVA the P values were all higher than 0.30). We thus grouped the two control treatments.

Nest-Manipulation significantly affected the number of chicks alive at ringing per nest (ANOVA with ordered expectations: Nest-Manipulation effect, $F_{1,135}$=25.86, P<

0.0001, Slope±SE=0.97±0.19): the number of chicks in the nest increased gradually from the −2 to the +2 Nest-Manipulation. These differences were maintained at fledging (ANOVA with ordered expectations: Nest-Manipulation effect, $F_{1,135}=17.55$, $P<0.0001$, Slope±SE=0.99±0.24, Fig. 1). The squared term of manipulation did not affect either to the number of chicks alive at ringing (ANOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,134}=0.49$, $P=0.47$) or to the number of fledged chicks (ANOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,134}=0.01$, $P=0.91$).

Chick body condition on capture day did not differ among Nest-Manipulation categories (ANCOVA: Nest-Manipulation effect, $F_{1,122}=0.02$, $P=0.90$). The squared term of manipulation did not affect to chick body condition on capture day either (ANCOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,123}=0.27$, $P=0.59$). Body condition was only related to the interaction between chicks' wing length and age (ANCOVA: interaction term wing length *age, $F_{1,124}=23.14$, $P<0.001$). No other interaction or main effect affected body mass (in ANCOVA the P values were all higher than 0.24).

Effects of nest-manipulation on parental investment

The two control groups did not differ in the global frequency of parental visits (one-way ANOVA: $F_{1,21}=0.44$, $P=0.52$), the frequency of parental visits per nestling (one-way ANOVA: $F_{1,21}=0.70$, $P=0.41$) or the rate of time spent by parents in the nest (one-way ANOVA: $F_{1,21}=1.95$, $P=0.18$). We thus grouped the two control treatments.

The global frequency of parental visits was positively related to the experimental brood size groups (ANOVA with ordered expectations: Nest-Manipulation effect, $F_{1,54}=5.49$, $P=0.02$, Slope±SE=4.59±1.96, Fig. 2): Nest-Manipulation affected parental number of visits, with increased broods receiving more visits. The squared term of Nest-Manipulation did not affect to this variable (ANOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,53}=0.84$, $P=0.365$). The frequency of parental visits per nestling was not affected either by Nest-Manipulation (ANOVA with ordered expectations: Nest-Manipulation effect, $F_{1,54}=0.50$, $P=0.48$) or by the squared term of Nest-Manipulation (ANOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,53}=0.20$, $P=0.65$). However, the rate of time spent by parents in the nests was not related to the Nest-Manipulation (ANOVA with ordered expectations: Nest-Manipulation effect, $F_{1,54}=0.16$, $P=0.69$), but it was negatively related to the squared term of Nest-Manipulation (ANOVA with ordered expectations: Squared Nest-Manipulation effect, $F_{1,54}=5.81$, $P=0.019$, Estimate±SE −46.2±19.2, Fig. 2). This result means that parents of control broods invested more time in their nests than parents having broods modified in one nestling and these ones more than the parents of highly modified broods (two nestlings moved).

Effects of nest-manipulation on probability of a second clutch

The two control groups did not differ in the probability of producing a second clutch (GLMM: $F_{1,44}=0.0$, $P=1.0$), and in both cases, there was a significant nest effect (GLMM: random effect of the nest nested in the patch, $Z=3.96$, $P<0.0001$). We thus grouped the two control treatments.

The probability of producing a second clutch during the season was inversely related to the intensity of the experimental Nest-Manipulation and also to laying dates of the first clutch (Table 2, Fig. 3), with the lowest probabilities for individuals from nests with increased brood sizes and later laying dates and higher ones for those in decreased and control nests and with earlier laying dates. The experimental Patch-Treatment significantly affected this probability too, as well as laying dates (Table 2). Furthermore, there was a Nest effect nested inside the Patch effect on the probability of producing a second clutch, i.e. this probability was not independent for the members of a same pair (Table 2). This is because the two pair members did not breed independently, a result supported by the fact that the divorce rate between first and second clutch in the population is only of 20%.

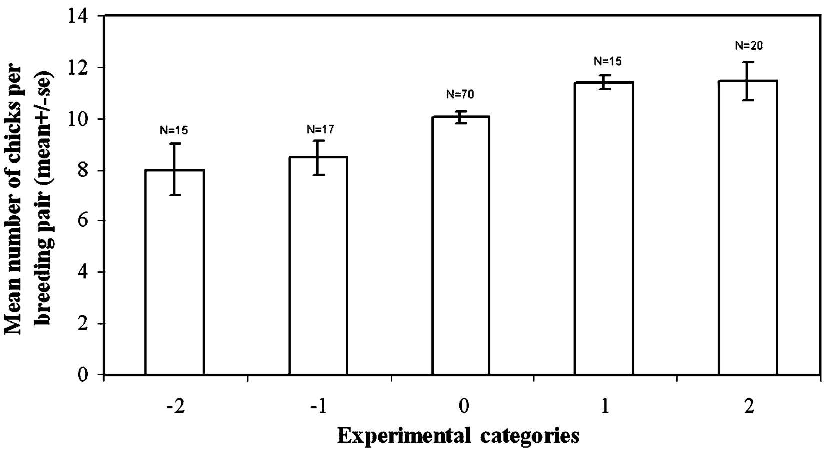
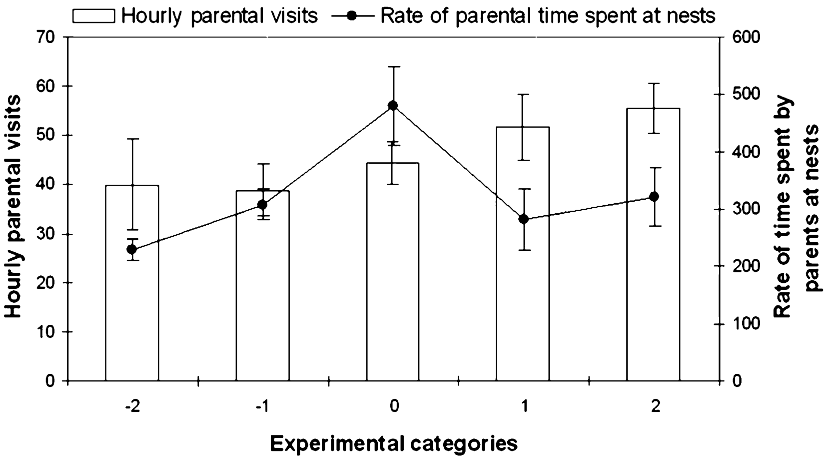


Fig. 1 Mean (±SE) number of fledglings per breeding blue tit pair in the experimentally reduced, enlarged and control broods. Experimental Nest-Manipulation involved the addition or removal of zero, one or two hatchlings. ANOVA with Nest-Manipulation as a fixed continuous effect and number of fledglings per breeding pair as the dependent variable ($P<0.0001$)

Fig. 2 Mean (±SE) hourly parental visiting frequency (summed feeding frequency of both sexes) and mean (±SE) rate of time spent by both parents inside nests to experimentally reduced, enlarged and control nests. Significant Nest-Manipulation effect on frequency of parental visits per breeding pair (P=0.02) and Nest-Manipulation squared effect on rate of time spent as the dependent variable (P=0.02)



Effects of nest-manipulation on parental mass, apparent local survival and recruitment

Male or female weight was not related to the Nest-Manipulation variable (one-ANOVA: Nest-Manipulation effect, $F_{5,105}$=1.13, P=0.35 and $F_{5,111}$=0.45, P=0.81 for males and females, respectively), thus indicating no short-term effect of the manipulation in the mass of parents. Apparent local survival (recorded as birds breeding in one of our patches in 2003 following our 2002 Nest-Manipulation) did not differ between the two control groups (GLMM, $F_{1,707}$= 0.05, P=0.82). In the control group only birds' age

Table 2 Summary of results of the effects of the brood size manipulation on variables measuring intra- and inter-generational reproductive costs in blue tits

Starting models					Results		
Dependent variable	Fixed effects	Random effects	Selected effects	Test	Statistic	P	Estimate±SE
Probability of a second clutch	NM	Nest (patch)	NM	GLMM	$F_{1,88}$=5.00	0.03	−2.03±0.91
	NM^2	Patch	Laying date		$F_{1,88}$=7.37	0.008	−1.44±0.53
	Laying date		Nest (patch)		Z=3.89	<0.0001	
	PT	Nest (patch)	PT	GLMM	$F_{1,88}$=4.23	0.043	−1.57±0.76
	PT^2	Patch	Laying date		$F_{1,88}$=7.04	0.0095	−1.48±0.56
	Laying date		Nest (Patch)		Z=5.71	<0.001	
Adult local survival	NM	Nest (patch)	Sex	GLMM	$F_{1,90}$=38.87	<0.001	
	Sex	Patch	Nest (patch)		Z=4.56	<0.001	
	NM*Sex						
	NM^2						
	PT	Nest (patch)	Sex	GLMM	$F_{1,89}$=38.96	<0.001	
	Sex	Patch	Nest (patch)		Z=4.57	<0.001	
	PT*Sex						
	PT^2						
Juvenile local survival	NM	Nest (patch)	Nest (patch)	GLMM	Z=4.75	<0.001	
	NM^2	Patch					
	PT	Nest (patch)	Nest (patch)	GLMM	Z=4.73	<0.001	
	PT^2	Patch					
Probability of local recruitment	NM		NM	Logistic regression model	χ_1^2=3.37	0.0662	0.34±0.19
	NM^2						
	PT		–	Logistic regression model	–	–	
	PT^2						

[a]Nest-Manipulation (NM) is included in models as a continuous variable taking integer values from −2 to +2. Similarly, Patch-Treatment (PT) is also considered as a continuous variable taking values from 1 to 3 (decreased to increased patches). Starting models are specified. Only effects with P values lower than 0.1 are shown. Slopes of continuous selected effects are shown

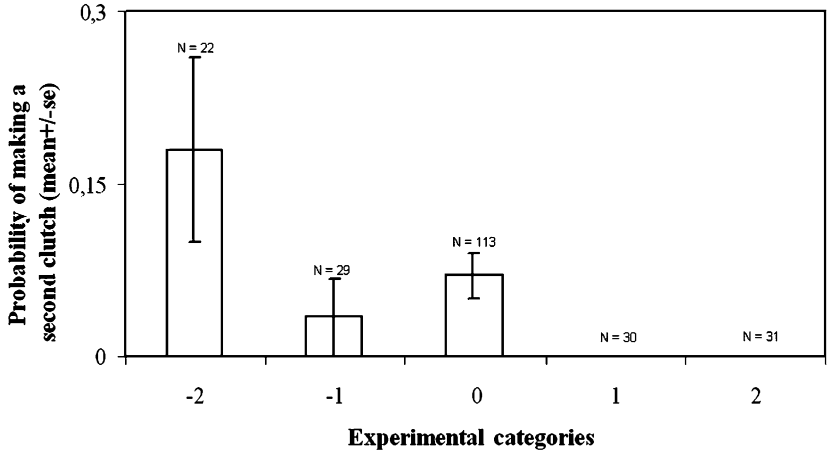


Fig. 3 Mean probability (±SE) to undertake a second reproduction during the season for individuals rearing experimentally reduced, enlarged and control broods. For significance of the effect and starting model, see Table 2

(GLMM, $F_{1,707}$=17.28, P<0.0001) and nest of origin (GLMM, random effect of the nest nested in the patch Z=2.14, P=0.02) affected apparent survival. We thus grouped the two control treatments.

Adult apparent local survival depended on sex, with higher apparent local survival for males (mean±SE, *N* 0.13±0.03, 111) than females (mean±SE, *N* 0.08±0.02, 117; Table 2). The Nest effect nested inside the Patch effect was also significant on (Table 2). No other tested effect significantly affected adult apparent local survival. Juvenile apparent local survival was only affected by the nest of origin: Nest effect nested inside the Patch effect (Table 2).

Local recruitment rate in 2003 was not influenced by the experimental Nest-Manipulation performed in the nest or the Patch-Treatment in 2002, although a near to significance positive relationship between local recruitment rate and Nest-Manipulation appeared (Table 2), involving that increased broods had higher probabilities of recruiting offspring than control or decreased broods. Nest- or Patch-Manipulation squared did not significantly affect these probabilities (Table 2). Mean (±SE, *N*=number of nests) numbers of recruits per brood and Nest-Manipulation category were 0.13 (±0.09, 15), 0.12 (±0.08, 17), 0.31 (±0.07, 70), 0.27 (±0.18, 15), 0.40 (±0.11, 20), respectively, for −2, −1, 0, 1, and 2 categories.

Discussion

Effect of our nest-manipulations

The experimental Nest-Manipulation affected offspring number positively. In fact, every time we added one hatchling, we got one fledging more (slope=0.99±0.24, Fig. 1). This shows that added hatchlings had a normal survival in the nest, implying that our Nest-Manipulations were not too strong so that adults could buffer it. Furthermore, body condition and number of parental visits per chick did not vary along our Nest-Manipulation gradient, showing that in spite of our Nest-Manipulation, each chick received, on average, the same amount of parental care. Additionally, there was no significant relationship between our gradient of manipulation of parental effort and recruitment rate, which supports the idea that breeders managed to maintain the quality of their offspring in spite of our Nest-Manipulations. These results suggest that parents compensated the effect of experimental Nest-Manipulation by increasing their investment in increased brood sizes and decreasing it in decreased nests. As expected, parental effort, measured as the frequency of parental visits, increased gradually with the intensity of our Nest-Manipulation. Also, the quadratic relationship found between time spent by parents inside nests and the Nest-Manipulation suggests that whilst parents of control broods devote the optimal time to their offspring, in decreased and increased broods, parents rest less time inside nests. The causes of this result in parents of decreased and increased broods are likely to differ: in decreased broods, parents probably do not need to stay many time inside nests because they have few offspring whilst in increased broods parents cannot devote many time to stay inside their nests because they responded to the manipulation increasing their number of visits to their nests, and thus, decreasing their free time to make other activities (Fig. 2). Thus, these results show that our brood size manipulation was effective and it did impose some real cost to breeders, though previous authors have questioned the efficiency of brood size manipulation in manipulating reproductive effort directly (Lessells 1991).

In testing predictions of the reproductive trade-off hypothesis by our Nest-Manipulation of blue tit brood size, we found that parental effort and fitness within the season were affected by that Nest-Manipulation. Parents of experimentally increased broods seem to pay the cost of an increased reproductive investment immediately after the end of the first breeding attempt because Nest-Manipulation decreased the occurrence of second clutches in increased relative to control or decreased groups, once controlled by the effect of laying date of the first clutch. We found no evidence supporting the existence of other intra-generational costs in a two-year period because adult weight and apparent adult local survival did not differ among treatments. Additionally, Nest-Manipulation did not imply any inter-generational costs because neither chicks' quality before fledging nor the probability of being recruited the next year in the population differed among Nest-Manipulation categories. Thus, we only found intra-generational costs of reproduction in blue tits, which agree with our expectations for such a short-lived bird.

Intra-generational costs of reproduction

We found that birds rearing enlarged broods were less likely to start a second clutch than those rearing control or decreased broods. Second clutches were relatively successful, with 5.11 fledglings on average. Thus, the benefit of a second brood exceeded that of raising two extra-chicks. Therefore, the "decision" of not doing a second clutch was probably not based in the trading of these two benefits. The observed effect could be caused either by some mechanism of physical exhaustion such as tissue damage due to high workload or by other of energy recovering such as the lack of energy to undertake a second reproduction by parents of increased broods compared to control or decreased broods. However, this second possibility seems less feasible because short-lived birds are not able, by definition, to store much energy. Similar results to ours were found in the closely related great tit *Parus major* (Tinbergen 1987; Tinbergen and Both 1999). However, no effects of brood size manipulations were found in other studies, both in this species (Sanz and Tinbergen 1999) or in others, either short (*Motacilla cinerea*, Klemp 2000) or long-lived (*Tyto alba*, Roulin et al. 1999) birds.

Although in birds decreases in mass related to the breeding effort are frequently reported (e.g. Nur 1984a; Moreno et al. 1995; Fargallo and Merino 1999), adult weight after manipulation was not related to our Nest-Manipulation in males or females. However, our measure of adult weight was performed between days 8 and 13 of the nesting period and perhaps the expected decrease in mass associated with reproductive effort might appear later in the season.

We found no evidence of long-term intra-generational reproductive costs in terms of reduced apparent local survival for parents rearing increased broods. However, our capacity to detect significant effects was probably weaker for long- than for short-term costs. The main reason of this lack of power is that our apparent local survival resulted from the product of four components that may vary independently: survival, dispersal, breeding and detection probabilities. It was not possible in our design to disentangle the effect of these components because many different scenarios can lead to the same apparent local survival. For instance, experimental Nest-Manipulation could affect dispersal decisions with birds rearing decreased broods being more prone to disperse than birds rearing increased or control nests as a consequence of their lower reproductive output. It has been shown that individual breeding success strongly affects dispersal probability (e.g., Danchin et al. 1998; Doligez et al. 1999; Schjørring et al. 2000). This scenario could produce the same apparent local survival along our gradient of Nest-Manipulation if survival probabilities diminished in birds with experimentally increased brood sizes. Although we cannot completely rule this possibility out, it is unlikely in this study because the study area is well isolated from other possible breeding areas by a lake and a large area of cultivated fields. Then, we would expect birds to disperse inside the same forest, which is large enough to provide many breeding opportunities. Indeed, 23 out of the 24 adult birds recaptured in 2003 changed nests (the only individual faithful to its nest had its brood size increased by two chicks), and four out of 24 changed patches from 2002 to 2003 (one being in the −1 and the other three in the 0 Nest-Manipulation groups), possibly suggesting some independence between dispersal decisions and Nest-Manipulation. Furthermore, for recaptured birds, dispersal distance between two reproductive events was not related either to Nest-Manipulation or to sex (ANOVA with ordered expectations: Interaction term, $F_{1,19}$=1.97, P=0.18; Nest-Manipulation effect, $F_{1,20}$=1.59, P=0.22; Sex effect, $F_{1,21}$=1.62, P=0.22), although the experimental manipulation might affect the dispersal probability and not the dispersal distance. Moreover, biologically meaningful survival differences between brood size manipulation categories might be so small as to remain undetected even in large samples (Roff 1992). This restriction seems to be maximal in short-lived birds because their survival rate is low (Sæther 1989). Thus, although we captured 76.9% of the breeders in 2003, the demonstration of the effects of the brood size manipulation upon parental survival seems to be difficult with our low recapture rate (only 24 recaptured individuals) in blue tits.

Adult apparent local survival was higher in males than females, in spite of a higher proportion of females captured on the nest for the 2 years of study. This result probably reveals higher dispersal proneness in females than males, which is the usual pattern in birds (Greenwood 1980) and more specifically in tits (Greenwood et al. 1979). Alternatively, this observation may result from a different incidence of reproductive costs in males than females, with females investing more in reproduction than males. However, we could not test this possibility because we did not record parental investment for the sexes separately.

Inter-generational costs of reproduction

Juvenile apparent local survival was affected by none of the variables tested, which is consistent with the fact that chick body condition was not affected by the experimental Nest-Manipulation. However, we found a significant effect of the nest of origin, implying that chicks from the same broods had similar fate.

Nest-Manipulation did not significantly affect the proportion of 2002 young locally recruited in 2003. This result suggests either the absence or the low importance of intergenerational effects of our Nest-Manipulation, which was of relatively small intensity. Alternatively, a differential proneness to philopatry of the offspring coming from nests having experienced manipulations of different types and intensities could also explain the result in conjunction with differential juvenile survival.

Nur (1984a,b), analysing reproductive costs in the blue tit, also found intra-generational costs of current reproduction (Table 1); however Pettifor (1993a,b) analysing his own data as well as re-analysing Nur's data, found little support for the occurrence of reproductive costs in the blue tit, measured either in terms of parental survival or future fecundity, and did not find any convincing explanation for the absence of these costs. Discrepancies with our results may result from the fact that Pettifor removed individuals that undertook a second clutch from his analyses (Pettifor 1993b). Other authors have provided evidence for the existence of both intra- and inter-generational costs of reproduction in the species (Table 1). One explanation of such discrepancies may be differences in the amplitude of the brood size manipulation (Tammaru and Hõrak 1999). In summary (Table 1), it seems desirable to account for intra- and inter-generational reproductive costs at the same time in future studies.

Most studies ignore the importance of non-breeding as a potential cost of reproduction or other behaviour such as dispersal (see comments in Danchin and Cam 2002). Only studies on three species analysed cost of reproductions in terms of probability of making a second clutch, thus accounting for non-breeding, and no other study accounted for it (Table 1). Our study is not totally immune to that question either. The reason is that in most cases, the biology and the experimental design do not allow the detection of non-breeders. However, missing one breeding opportunity constitutes a major cost, particularly in short lived species. In the present study, we study costs of reproduction in terms of the second breeding opportunity during the season and thus account for non-breeding in that population. However, the fact that only 7.08% of the birds normally lay second clutches in our population reduces the importance of non-breeding during the second clutch because most individuals don't undertake second clutches anyway. More generally, it seems important to account for non-breeding birds in the population because non-breeding may reveal costs of previous activities. Thus, cavity-nesting birds are unlikely to be the best biological models to study the costs of reproduction, as long as we cannot detect the non-breeding fragment of the population (Danchin and Cam 2002; see however Orell and Belda 2002).

Acknowledgements We thank all people who collaborated in data collection: M. Diot, A. Dreiss, I. Dworzynska, M. Geier, J. Pater, M. Xavier, J. White and many others. J. Avilés provided useful comments on the manuscript. Two anonymous referees also provided interesting suggestions to the manuscript. D. Parejo was supported by a Postdoctoral Marie Curie Fellowship (contract HPMF-CT-2000-00716). All procedures applied here comply with the French laws.

References

Askenmo C (1979) Reproductive effort and return rate of male pied flycatchers. Am Nat 114:748–753

Bell G, Koufopanou V (1986) The cost of reproduction. In: Dawkins R, Ridley M (eds) The Cost of Reproduction. Oxford University Press, Oxford, pp 83–131

Blondel J, Maistre M, Perret P, Hurtrez-Boussés S, Lambrechts MM (1998) Is the small clutch size of Corsican blue tit population optimal? Oecologia 117:80–89

Boyce MS, Perrins CM (1987) Optimizing great tit clutch size in a fluctuating environment. Ecology 68:142–153

Cramp S, Simmons KEL (eds) (1988) The Birds of the Western Palearctic, vol. V. Oxford University Press, Oxford

Daan S, Deerenberg C, Dijkstra C (1996) Increased daily work precipitates natural death in the kestrel. J Anim Ecol 65:539–544

Danchin E, Cam E (2002) Can non-breeding be a cost of breeding dispersal? Behav Ecol Sociobiol 51:153–163

Danchin E, Boulinier T, Massot M (1998) Breeding habitat selection based on conspecific reproductive success: implications for the evolution of coloniality. Ecology 79:2415–2428

Danchin E, Heg D, Doligez B (2001) Public information and breeding habitat selection. In: Clobert J, Danchin E, Dhondt AA, Nichols JD (eds) Dispersal. Oxford University Press, Oxford, pp 243–258

Danchin E, Giraldeau L-A, Valone TJ, Wagner RH (2004) Public information: from nosy neighbors to cultural evolution. Science 305:487–491

Deerenberg C, Penn I, Dijkstra C, Arkies BJ, Visser, GH, Daan S (1995) Parental energy expenditure in relation to manipulated brood size in the European kestrel *Falco tinnunculus*. Zoology 99:39–48

De Steven D (1980) Clutch-size, breeding success, and parental survival in the tree swallow (*Iridoprocne bicolour*). Evolution 34:278–291

Dijkstra C, Bult A, Bijlsma S, Daan S, Meijer T, Zijlstra M (1990) Brood size manipulations in the kestrel (*Falco tinnunculus*): effects on offspring and parent survival. J Anim Ecol 59:269–285

Doligez B, Danchin E, Clobert J, Gustafsson L (1999) The use of conspecific reproductive success for breeding habitat selection in a non-colonial, hole-nesting species, the collared flycatcher. J Anim Ecol 68:1–15

Drent RH, Daan S (1980) The prudent parent: energetic adjustments in avian breeding. Ardea 80:225–252

Fargallo JT, Merino S (1999) Brood size manipulation modifies the intensity of infection by Haematozoa in female Blue Tits *Parus caeruleus*. Ardea 87:261–268

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Anim Behav 28:1140–1162

Greenwood PJ, Harvey PH, Perrins CM (1979) The role of dispersal in the Great Tit (*Parus major*): the causes, consequences and heritability of natal dispersal. J Anim Ecol 48:123–142

Gustafsson L, Sutherland WJ (1988) The costs of reproduction in the collared flycatcher *Ficedula albicollis*. Nature 335:813–815

Harris MP (1966) Breeding biology of manx shearwaters. Ibis 108:17–33

Harris MP (1970) Breeding ecology of the swallow-tailed gull *Creagrus furcatus*. Auk 87:215–243

Hegner RE, Wingfield JC (1987) Effects of brood-size manipulations on parental investment, breeding success, and reproductive endocrinology of house sparrows. Auk 104:470–480

Hõrak P (2003) When to pay the cost of reproduction? A brood size manipulation experiment in great tits (*Parus major*). Behav Ecol Sociobiol 54:105–112

Hõrak P, Ots I, Murumagi A (1998) Haematological health state indices of reproducing Great Tits: a response to brood size manipulation. Funct Ecol 12:750–756

Jacobsen KO, Erikstad KE, Saether BE (1995) An experimental study of the costs of reproduction in the kittiwake *Rissa tridactyla*. Ecology 76:1636–1642

Jarvis MFJ (1974) The ecological significance of clutch size in the South African gannet (*Sula capensis*). J Anim Ecol 43:1–17

Kluyver HN (1951) The population ecology of the Great Tit, *Parus major*. Ardea 39:1–135

Klemp S (2000) Effects of parental effort on second brood, moult and survival in the Grey Wagtail *Motacilla cinerea*. Ardea 88: 91–98

Korpimäki E (1988) Costs of reproduction and success of manipulated broods under varying food conditions in Tengmalm's owl. J Anim Ecol 57:1027–1039

Lessells CM (1991) The evolution of life histories. In: Krebs JR, Davies NB (eds.), Behavioural ecology: an evolutionary approach. Blackwell, Oxford, UK, pp 32–68

Lindén M (1988) Reproductive trade-off between first and second clutches in the great tit *Parus major*: an experimental study. Oikos 51:285–290

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS System for Mixed Models. SAS Institute, Cary, USA

Merilä J, Andersson M (1999) Reproductive effort and success are related to haematozoan infections in blue tits. Ecoscience 6:421–428

Merilä J, Wiggings DA (1997) Mass loss in breeding blue tits: the role of energetic stress. J Anim Ecol 66:452–460

Moreno J, Cowie RJ, Sanz JJ, Willians SRS (1995) Differential response by males and females to brood manipulations in the pied flycatcher: energy expenditure and nestling diet. J Anim Ecol 64:721–732

Moreno J, Sanz JJ, Arriero E (1999) Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. Proc R Soc Lond B 266: 1105–1109

Nur N (1984a) The consequences of brood size for breeding blue tits I. Adult survival, weight change and the cost of reproduction. J Anim Ecol 53:479–496

Nur N (1984b) The consequences of brood size for breeding blue tits II. Nestling weight, offspring survival and optimal brood size. J Anim Ecol 53:497–517

Nur N (1988) The consequences of brood size for breeding blue tits III. Measuring the cost of reproduction, survival, future fecundity, and differential dispersal. Evolution 42:351–362

Orell M, Belda EJ (2002) Delayed cost of reproduction and senescence in the willow tit *Parus montanus*. J Anim Ecol 71:55–64

Orell M, Koivula K (1988) Cost of reproduction: Parental survival and production of recruits in the willow tit *Parus montanus*. Oecologia 77:423–432

Orell M, Koivula K, Rytkönen S, Lahti K (1994) To breed or not to breed: causes and implications of non-breeding habit in the willow tit *Parus montanus*. Oecologia 100:339–346

Orell M, Rytkönen S, Koivula K, Ronkainen M, Rahiala M (1996) Brood size manipulations within the natural range did not reveal intragenerational cost of reproduction in the willow tit *Parus montanus*. Ibis 138:630–637

Perrins CM, Moss D (1975) Reproductive rates in the great tit. J Anim Ecol 44:695–706

Pettifor RA, Perrins CM, McCleery RH (1988) Variation in clutch size in great tits: evidence for the individual optimalization hypothesis. Nature 336:160–162

Pettifor RA (1993a) Brood manipulations experiments. I. The number of offspring surviving per nest in blue tits (*Parus caeruleus*). J Anim Ecol 62:131–144

Pettifor RA (1993b) Brood-manipulation experiments. II. A cost of reproduction in blue tits (*Parus caeruleus*). J Anim Ecol 62: 145–159

Reid WV (1987) The cost of reproduction in the glaucous-winged gull. Oecologia 74:458–467

Roff DA (1992) The evolution of life histories. Theory and analysis. Chapman and Hall, London, UK

Røskaft E (1985) The effect of enlarged brood size on the future reproductive potential of the rook. J Anim Ecol 54:255–260

Roulin A, Ducrest AL, Dijkstra C (1999) Effect of brood size manipulations on parents and offspring in the Barn Owl *Tyto alba*. Ardea 87:91–100

Rytkönen S, Orell M (2001) Great tits, *Parus major*, lay too many eggs: experimental evidence in mid-boreal habitats. Oikos 13:439–450

Saino N, Calza S, Ninni P, Moller, AP (1999) Barn swallows trade survival against offspring condition and inmunocompetence. J Anim Ecol 68:999–1009

Sanz JJ (1997) Clutch size manipulation in the Pied Flycatcher: effects on nestling growth, parental care and moult. J Avian Biol 28:157–162

Sanz JJ, Tinbergen JM (1999) Energy expenditure, nestling age, and brood size: an experimental study of parental behavior in the great tit *Parus major*. Behav Ecol 10:598–606

SAS (1999) SAS user's guide. Version 8 edition. SAS Institute

Schjørring S, Gregersen J, Bregnballe T (2000) Sex difference in criteria determining fidelity towards breeding sites in the great cormorant. J Anim Ecol 69:214–223

Stearns SC (1992) The evolution of life histories. Oxford Univ Press, Oxford

Stjernman M, Raberg L, Nilsson JA (2004) Survival costs of reproduction in the blue tit (*Parus caeruleus*): a role for blood parasites? Proc R Soc Lond B 271:2387–2394

Sæther B-E (1989) Survival rates in relation to body weight in European birds. Ornis Scand 20:13–21

Tammaru T, Hõrak P (1999) Should one invest more in larger broods? Not necessarily. Oikos 85:574–581

Tinbergen JM (1987) Costs of reproduction in the great tit: intraseasonal costs associated with brood size. Ardea 75:111–122

Tinbergen JM, Both C (1999) Is clutch size individually optimised? Behav Ecol 10:504–509

Tinbergen JM, Daan S (1990) Family planning in the great tit (*Parus major*): optimal clutch size as integration of parent and offspring fitness. Behaviour 114:161–190

Tinbergen JM, Verhulst S (2000) A fixed energetic ceiling to parental effort in the great tit? J Anim Ecol 69:323–334

Velando A, Alonso-Alvarez C (2003) Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. J Anim Ecol 72:846–856

Verhulst S 1995 Reproductive decisions in great tits. An optimally approach. Ph.D. Thesis, University of Groningen

Verhulst S, Hut RA (1996) Post-fledging care, multiple breeding and the costs of reproduction in the great tit. Anim Behav 51:957–966

Verhulst S, Tinbergen JM (1997) Clutch size and parental effort in the great tit *Parus major*. Ardea 85:111–126

Willians GC (1966) Natural selection, the costs of reproduction and a refinement of Lack's principle. Am Nat 100:687–690