

No experimental evidence for local competition in the nestling phase as a driving force for density-dependent avian clutch size

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Summary

1. In birds, local competition for food between pairs during the nestling phase may affect nestling growth and survival. A decrease in clutch size with an increase in breeding density could be an adaptive response to this competition. To investigate whether breeding density causally affected the clutch size of great tits (*Parus major*), we manipulated breeding density in three out of eight study plots by increasing nest-box densities. We expected clutch size in these plots to be reduced compared to that in control plots.

2. We analysed both the effects of variation in annual mean density (between-year comparisons) and experimental density (within-year comparison between plots) on clutch size variation, the occurrence of second broods and nestling growth. We examined within-female variation in clutch size to determine whether individual responses explain the variation over years.

3. Over the 11 years, population breeding density increased (from 0.33 to 0.50 pairs ha⁻¹) while clutch size and the occurrence of second broods decreased (respectively from 10.0 to 8.5 eggs and from 0.39 to 0.05), consistent with a negative density-dependent effect for the whole population. Nestling growth showed a declining but nonsignificant trend over years.

4. The decline in population clutch size over years was primarily explained by changes occurring within individuals rather than selective disappearance of individuals laying large clutches.

5. Within years, breeding density differed significantly between manipulated plots (0.16 pairs ha⁻¹ vs. 0.77 pairs ha⁻¹) but clutch size, occurrence of second broods and nestling growth were not affected by the experimental treatment, resulting in a discrepancy between the effects of experimental and annual variation in density on reproduction.

6. We discuss two hypotheses that could explain this discrepancy: (i) the decline in breeding performance over time was not due to density, but resulted from other, unknown factors. (ii) Density did cause the decline in breeding performance, but this was not due to local competition in the nestling phase. Instead, we suggest that competition acting in a different phase (e.g. before egg laying or after fledgling) was responsible for the density effect on clutch size among years.

Key-words: breeding density, great tit, individual adjustment, *Parus major*, reproduction

Introduction

Density dependence is a key concept in ecology, since it explains a large part of the regulation of populations (e.g. Murdoch 1994). Demographic traits that are affected by population density include dispersal, survival and reproduction (Sinclair 1989; Newton 1998). The existence of density

dependence of avian reproduction is strongly supported by descriptive time-series analyses (e.g. Both 2000; Wilkin *et al.* 2006), yet little is known regarding the resources that birds must compete for and thus about the mechanisms involved in density-dependent processes. Experiments that study the causality of density-dependent relationships by manipulating density have produced inconsistent results (see review in Newton 1998). Some of these experiments found a causal relationship between density and demographic traits like

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survival (Verhulst 1992) and reproduction (Kluijver 1951; Dhondt, Kempenaers & Adriaensen 1992; Both 1998b; Sillett, Rodenhouse & Holmes 2004), but others failed to find a causal link (Tompa 1967; Both & Visser 2000). This discrepancy suggests that the effects of density on demographic traits depend on the ecological circumstances of these different populations.

Competition for limited resources (e.g. territories, mates, and food) is often hypothesized as one of the main mechanisms behind the density dependence of reproduction (Newton 1998). The strength and the type of competition may differ between the different phases of the annual cycle depending on fluctuation in the availability of resources and the number of competitors.

Competition for food before or during egg laying may affect the amount of energy allocated to reproduction (Lack 1966) and could cause density dependence of reproduction. Experimental food supplementation during egg laying did increase clutch size and offspring production in a number of species (Arcese & Smith 1988; Soler & Soler 1996; Nager, Ruedger & van Noordwijk 1997) supporting this idea.

Breeding density manipulations have also revealed that reproductive performance of birds generally decreased in experimental high-density plots (Török & Toth 1988; Both 1998b). The observation that not only clutch size but also nestling growth was causally related to local densities suggests that nestlings in high-density areas received less food. Therefore, local competition for food during the nestling phase might be another mechanism causing birds to adjust their reproductive rates (Both 1998b).

Finally, females may produce a number of offspring adjusted to the level of competition they will encounter after fledging. If the density of birds is such that the level of competition is raised, this decreases the chance for juveniles to become recruited into the local population (Drent 1984). Therefore, the benefits of producing fewer but more competitive offspring may increase at high densities. Experiments involving removal of breeding pairs showed that the newcomers were mainly young birds and that recolonization occurred often to the original population level, implying that the amount of available resources (including space to establish territories) limits local densities (e.g. Watson & Jenkins 1968; Krebs 1970; but see Marra & Holmes 1997).

Variation in phenotypic traits is common in birds and population changes in those traits can occur as a result of within-individual changes or selective appearance or disappearance of certain phenotypes. Several studies on avian clutch size have shown that individuals tune their clutch size to both their local situation and their phenotypic quality so that it maximizes their fitness (individual optimization hypothesis; Pettifor, Perrins & McCleery 1988; Tinbergen & Daan 1990; Pettifor, Perrins & McCleery 2001), although not under all circumstances (Both, Tinbergen & Van Noordwijk 1998; Tinbergen & Both 1999; Tinbergen & Sanz 2004). Both *et al* (2000) also estimated that the fitness consequences of clutch size changed with population density, shifting the clutch size with maximum fitness (optimal clutch size) to

lower values at higher breeding densities. Because the negative effect of family size on nestling growth was stronger at higher population densities, the authors suggested that a reduction of clutch size with density was generated by local competition for nestling food.

In studies on great tits (*Parus major*), where most of the evidence for density dependence of avian clutch size comes from (see review in Both 2000), it is usually assumed that breeding birds compete for caterpillars to feed to their offspring, and that what drives density-dependent patterns in reproduction is the depletion of these caterpillars. However, in different ecological circumstances, there may be no competition for food during the chick-rearing phase, and a density manipulation under these conditions may allow the detection of alternative mechanisms that can cause density dependence.

In this study, we investigated causal effects of breeding density on clutch size and nestling growth in great tits, studying experimental density effects within years in concert with natural density effects between years. We performed a density experiment for 11 years in a growing population in the north of the Netherlands where flying insects rather than caterpillars are an important component of the diet (personal observations). Woodlots carrying either a low or high density of nest boxes were created. This experimental set up allowed us to analyse simultaneously the effect of the local experimental density (within-year comparison) and the effect of natural annual breeding density variation (between-year comparisons) on reproduction in great tits. If local competition for food is the mechanism behind the density dependence of clutch size, local high densities are expected to negatively influence clutch size, nestling growth and the probability of having a second brood, as observed in another great tit population (Both 1998b). If birds can or have to adjust their clutch size between breeding attempts as a response to changes in annual conditions ('individual adjustment'; Both 1998a), we expect the relationship between population clutch size and annual breeding density to result from within-individual changes. In contrast, if the relationship between population clutch size and annual breeding density results from the selective appearance or disappearance of certain phenotypes, selection may be involved.

Material and methods

STUDY AREA AND STUDY SPECIES

The study was carried out on a great tit population living in a mixed deciduous forest in the Lauwersmeer area (53°20' N, 06°12' E), a region in the north of the Netherlands that was reclaimed from the Wadden Sea in 1968 (see details in Tinbergen & Sanz 2004; Tinbergen 2005). At the start of the study in 1993, 80 nest boxes were present, and 120 boxes were added from 1994 onwards. Few natural cavities were available in the study area so that virtually all the breeding attempts occurred in the nest boxes. In our population, great tits laid on average 9.33 (SD = 1.71, $n = 1209$, 1994–2004) eggs for the first clutches and 7.43 (SD = 1.32, $n = 320$) eggs for the second clutches.

DENSITY EXPERIMENT

In 1994, five plots with low nest-box densities (from 0.11 to 0.83 nest-boxes ha^{-1}) and three plots with high nest-box densities (from 1.25 to 1.64 nest-boxes ha^{-1}) were created (Fig. 1). We determined the experimental densities, especially those of the high-density plots, based on the natural range of breeding densities recorded in other Dutch great tit populations where negative density dependence of clutch size at a population level was found (Both 1998a, see also Fig. 7). Plots were spread over two main woodlots ('north' or 'south' areas) about 3.5 km apart. Plots were all physically separated by open grass areas or woodlands that did not carry any nest boxes, except for the two adjacent plots 1 and 2 (Fig. 1). The shortest distance between any nest box from plot 1 and plot 2 was 107 m. We thus consider all the plots as being isolated and did not treat females breeding on the edge of a plot differently from those breeding closer to the centre of a plot.

Ideally, we would have switched the density treatments between plots over time to control for clutch size differences between plots. However, we did not do this because it would conflict with other aspects of our research. Therefore, we checked whether clutch size in 1993, before the density treatment (when all plots still had low breeding densities), covaried with the later plot treatment. We did this using a mixed model where plots and females were fitted as random effects and geographical location (north/south) and later experimental density treatment (low/high) were fitted as fixed effects. This analysis revealed that in 1993, the plots in the north had smaller clutches than plots in the south. Clutch size, however, did not covary with the later density treatment (mixed model: intercept: -0.74 ± 0.26 ; location: $\beta = 1.29 \pm 0.33$, $\chi^2 = 15.46$, d.f. = 1, $P < 0.001$; experimental treatment: $\beta = 0.51 \pm 0.32$, $\chi^2 = 2.58$, d.f. = 1, $P = 0.108$). The density treatment for each plot was thus sufficiently uncorrelated with respect to the original clutch size over the plots.

We defined local breeding density as the number of breeding pairs per surface area (pairs/hectare) for each plot with either low- or



Fig. 1. Map of the study area with the eight nest-box plots. Dark grey areas are wooded areas without nest boxes. Light grey areas represent water. Woodlots 1, 3, 5, 6 and 8 carried a low density of nest boxes and woodlots 2, 4 and 7 carried a high density of nest boxes.

high-density treatment. Annual breeding density was defined as the number of breeding pairs per surface area (pairs/hectare) in the whole study area. Annual breeding densities were separated into first brood breeder densities and second brood breeder densities (see definition of first and second broods in the section 'data selection') and were calculated for every study year.

DATA COLLECTION

From the beginning of April, nest boxes were checked weekly and parameters such as laying date (date of the first egg in the nest, back-calculated assuming that one egg was laid per day) and clutch size were monitored. Before expected hatching, nest boxes were checked daily to determine hatching date (day 0). At day 7, all chicks were ringed and both parents were caught with a spring trap in the nest box, measured (wing length, tarsus length and mass) and ringed for identification if necessary. At day 14, juveniles were weighed (mass ± 0.1 g) and measured (tarsus ± 0.1 mm and length of the third primary feather from the outside ± 0.5 mm). First brood averages for juveniles between 1994 and 2004 were 16.6 ± 1.7 g for mass, 19.6 ± 0.8 mm for tarsus length and 33.0 ± 4.2 mm for wing length ($n = 3859$).

DATA SELECTION

We used data from 11 years (1994 to 2004) of the study, with the exception of the nestling growth parameters, for which 10 years (1995 to 2004) were available. First broods were defined as clutches started within 30 days after the start of the earliest clutch in that year. For investigations at the population level, second broods were also analysed, excluding repeat clutches after failure of the first clutch. Second broods were thus defined as broods laid by females that were known to have successfully fledged a first brood. Clutches were excluded from the analysis when the female was unknown, or when they were smaller than 5 eggs and larger than 15 eggs because these are often the result of a disturbed situation (desertion or multiple females laying in the same box). For nestling growth and within-female analyses, nests with clutch size or brood size manipulations or manipulation of the parents were excluded from the analyses.

DATA ANALYSIS

Clutch size and nestling growth

The analyses of clutch size and nestling growth were conducted in two main steps. First, to account for sources of interdependency between measurements, models based on hierarchical data structure distinguishing between variance on two or three levels (mixed models) were built using multilevel modelling in MLWIN version 2.02 (Rasbash *et al.* 2004). Second, the year estimates given by these models were regressed on annual breeding density using STATISTICA version 7 (StatSoft Inc. 2004). Significance level was set at $P < 0.05$.

For clutch size (CS) and the probability of producing a second clutch, variation was estimated simultaneously at a plot level (denoted i) where the density experiment was performed, at the female level (denoted ij) grouped within plots, and at the within-female level (denoted ijk) (equations 1 and 3). This was carried out because several females bred repeatedly in the same plot in different years. For the nestling growth parameters (NGP), variation at a plot level (denoted i), variation at a brood level (denoted ij) and variation at an individual chick level (denoted ijk) were estimated simultaneously

(equation 2). The dependent variables were standardized by subtracting from the individual trait value the mean trait value of the population calculated for all the years together and then dividing this difference by the standard deviation of the population trait. All the explanatory variables that were continuous were centred on the population mean by subtracting the mean trait value of the population from the individual trait value. We used normal response models because clutch size and nestling growth parameters were distributed normally. We tested the significance of the explanatory variables 'experimental density treatment' ('exp. treat.': low or high), 'location' ('loc.': north or south), 'year' (categorical variable) and interactions with year (year \times loc. and year \times exp. treat.) in the models (equations 1 and 3). We included geographical location because the two areas differed in their soil structure, which is important for the quality of habitat and can explain part of the clutch size variation. The random effects regression models for clutch size variation tested were as follows:

$$CS_{ijk} = \beta_0 + \beta_1 * \text{exp. treat.} (0/1)_i + \beta_2 * \text{loc.} (0/1)_i + \beta_{3-13} * \text{year}_{ijk} + \beta_{14-34} * \text{interactions}_{ijk} + u_{0i} + e_{0ij} + v_{0ijk} \quad (\text{eqn 1})$$

All the β 's represent the fixed parts of the model while u_{0i} , e_{0ij} and v_{0ijk} are the random parts of the model and refer to the errors at each level of variation denoted i , ij and ijk . Experimental density is considered to have an effect on CS if β_1 is significantly different from 0.

For the descriptive analysis of natural density on clutch size, we regressed the annual estimates for clutch size controlled for treatment, location and interactions with year in this model on the annual breeding density. We used the annual breeding density of the first brood breeders to explain the variation in first clutches and the annual breeding density of the first as well as the second brood breeders to explain the variation in second clutches.

For nestling growth, the year estimates were controlled for clutch size, because chicks from larger broods usually grow less well:

$$NGP_{ijk} = \beta_0 + \beta_1 * \text{exp. treat.} (0/1)_i + \beta_2 * \text{loc.} (0/1)_i + \beta_{3-13} * \text{year}_{ij} + \beta_{14} * \text{clutch size}_{ij} + \beta_{15-33} * \text{interactions}_{ij} + u_{0i} + e_{0ij} + v_{0ijk} \quad (\text{eqn 2})$$

Probability of having a second clutch

To analyse the probability of producing a second clutch in relation to variation in density, we used a binomial response model with a logit link function based on hierarchical data structure distinguishing between variance at a plot level (denoted i), at a female level (denoted ij) and at a within female level (denoted ijk). As for the previous models, we tested the significance of the explanatory variables 'experimental density treatment' ('exp. treat.': low or high), 'location' ('loc.': north or south) and 'year' (categorical variable) in the model:

$$P(\text{second clutch}) = \beta_0 + \beta_1 * \text{exp. treat.} (0/1)_i + \beta_2 * \text{loc.} (0/1)_i + \beta_{3-13} * \text{year}_{ijk} + u_{0i} + e_{0ij} + v_{0ijk} \quad (\text{eqn 3})$$

We were not able to test the interactions with year due to an overparameterization of the model. The year estimates for the probability of having a second brood size controlled for treatment, location and year in this model were then regressed on the annual breeding density of early breeders after a back transformation $\exp(z)/[1 + \exp(z)]$ (z being equation 3).

Laying date

The effect of local and annual year density on laying date was tested the same way as for clutch size. Laying date did present significant

year variation; however, it did not significantly covary with local experimental density nor with annual year density (results not shown). Thus, results concerning laying date will not be discussed here and we will focus on the effect of density on clutch size and growth parameters.

Within-female analysis

With this analysis, we tested to what extent the change in mean annual clutch size can be statistically explained by individual adjustments of clutch size to density. The analyses of clutch size variation (CS) within individuals was carried out using a normal response model based on hierarchical data structure distinguishing between variance at the between-individual level (denoted i) and the within-individual level (denoted ij). The structure of the models took into account the fact that measurements for the same individual were not independent. For the analyses, female clutch sizes were standardized and the density values centred on the population mean. We used all first brood breeding females in the analysis including birds that bred only once.

We included annual breeding density (D) in the model to test for individual adjustment in relation to a change in year density (variation within individuals; level ij) as well as the quadratic term D^2 to test for a nonlinear effect of annual breeding density. We also incorporated the mean lifetime density experienced by a female (mD) to test for between-individual changes (level i) as well as the age of the female (α) and its quadratic term (α^2) (level ij) to correct for an effect of senescence known in the great tit (Kluyver 1951). Age of the females was included as a continuous variable.

The significance of the explanatory variables 'annual breeding density (D)', 'mean lifetime density (mD)', 'age' (α) and the quadratic terms ' D^2 ' and ' α^2 ' was tested in the model (equation 4) as follows:

$$CS_{ij} = \beta_0 + \beta_1 * D_{ij} + \beta_2 * \alpha_{ij} + \beta_3 * mD_i + \beta_4 * D_{ij}^2 + \beta_5 * \alpha_{ij}^2 + u_{0i} + e_{0ij} \quad (\text{eqn 4})$$

As for the previous models, all the β 's are the fixed parts of the model and u_{0i} and e_{0ij} are the random parts of the model.

Results

EXPERIMENTAL LOCAL DENSITY VS. ANNUAL BREEDING DENSITY

Local breeding density differed significantly between the two density treatments (paired t -test across years: $t = -20.85$, d.f. = 10, $P < 0.01$) meaning that our experiment did affect the distribution of the birds. In the low nest-box density areas, the local breeding density was on average 0.17 pairs ha⁻¹ (SD = 0.02, $n = 11$), compared to an average breeding density of 0.77 pairs ha⁻¹ in the high-density nest-box areas (SD = 0.01, $n = 11$). Between years, local breeding densities were positively correlated between the treatments ($r = 0.71$), suggesting that other factors than the local nest-box density also affected the local breeding densities. Nest-box occupancy rate differed markedly between the density treatments (paired t -test: $t = 8.02$, d.f. = 10, $P < 0.01$), with a higher occupancy in the low-density treatment (mean occupation rate low density: 69.14% SD = 10.26; high density: 51.69% SD = 7.52, $n = 11$). Furthermore, variance in local breeding densities also differed between treatments, with lower variance in the low nest-box

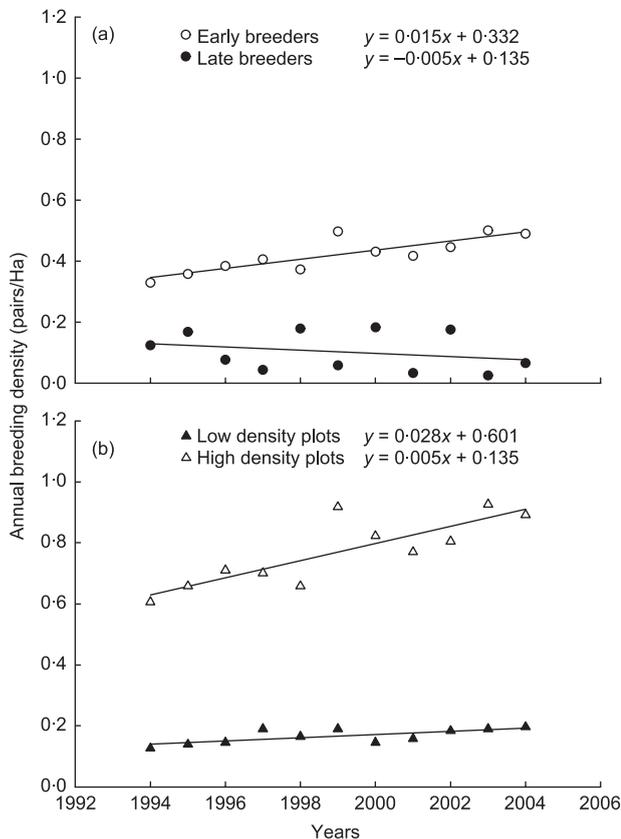


Fig. 2. (a) Variation in the annual density of first and second brood breeders between years. (b) Variation in the annual density of birds breeding in low- and high-density plots over the 11 years of the study.

group (variance low density: 0.001; high density: 0.012), probably because of the higher nest-box occupancy.

We found that the local breeding density of the first brood breeders, but not of the second brood breeders, significantly increased over the course of our 11-year study [group (first/second brood breeders): $F_{1,22} = 17.72$, $P < 0.01$; years: $F_{1,22} = 1.94$, $P = 0.180$; group \times years: $F_{1,22} = 8.64$, $P < 0.01$; Fig. 2a.]. We also found that the local breeding densities of both low- and high-density plots significantly increased with years in a similar way (using log-densities: density treatment (low/high): $F_{1,22} = 280.94$, $P < 0.01$; years: $F_{1,22} = 28.08$, $P < 0.01$; density treatment \times years: $F_{1,22} = 0.09$, $P = 0.762$, Fig. 2b).

CLUTCH SIZE – FIRST BROODS

Clutch size was slightly higher in the low-density treatment, but the difference was far from significant. (Table 1; Fig. 3a). It, however, did vary significantly with year (but not with treatment \times year), location and location \times year (Table 1). The clutch size estimates obtained for each year and controlled for treatment, location and for the year interactions did correlate negatively with the annual breeding density of the first brood breeders. This correlation was significant for both low- and high-density plots (all: $F_{1,11} = 18.91$, $P < 0.01$, Fig. 3b; low-density plots: $F_{1,11} = 33.83$, $P < 0.01$; high-density plots: $F_{1,11} = 13.83$, $P < 0.01$).

CLUTCH SIZE – SECOND BROODS

Clutch size of second broods did not differ significantly between experimental density treatments and did not significantly vary with location, year and the interactions with year (Table 1). The clutch size estimates obtained for each year and controlled for location, experimental treatment and the year interactions did not significantly relate to the annual density of the first brood breeders. This remained the case when looking at low- and high-density treatments separately (all: $F_{1,11} = 0.19$, $P = 0.675$; low-density plots: $F_{1,11} < 0.001$, $P = 0.985$; high-density plots: $F_{1,11} = 0.22$, $P = 0.647$). The clutch size estimates, however, positively correlated with the annual density of second brood breeders. This correlation was significant for the high-density plots only (all: $F_{1,11} = 6.13$, $P = 0.035$; low-density plots: $F_{1,11} = 0.20$, $P = 0.660$; high-density plots: $F_{1,11} = 11.56$, $P < 0.01$).

PROBABILITY OF HAVING A SECOND BROOD

The probability of having a second brood was not affected by the experimental density treatments, nor by the location. It differed significantly however between years (Table 2). The probability estimates back-transformed and controlled for

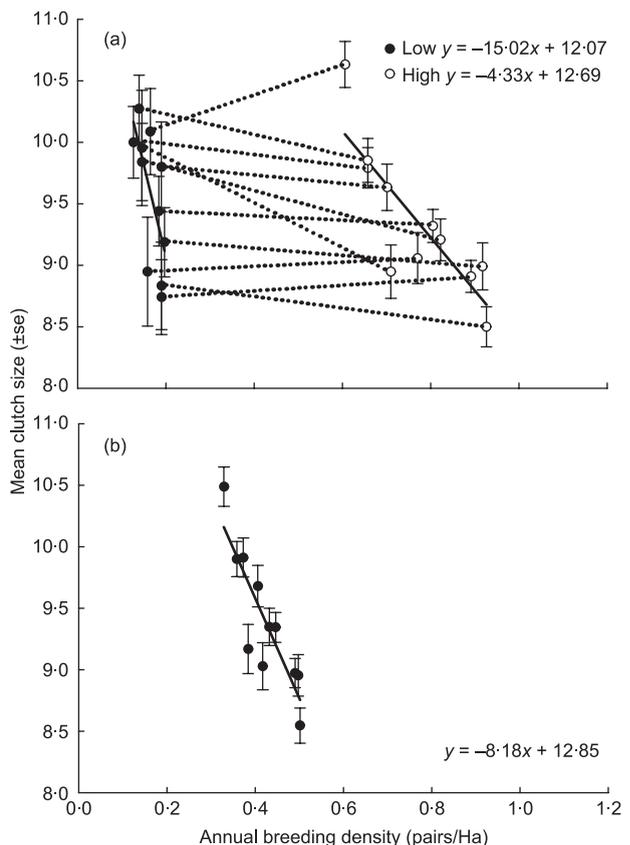


Fig. 3. (a) Within-year relationship between the mean population clutch size (\pm SE) and the local breeding density treatments (low density: closed circles; high density: open circles). (b) Relationship between the mean population clutch size (\pm SE) and the annual breeding density over the years.

Table 1. Model summary examining clutch size of the great tit for first ($n = 1119$ females) and second broods ($n = 267$ females) in relation to experimental density treatment, location (north or south), year, experimental density treatment \times year and location \times year. Significant values are shown in bold

Parameter	Standardized clutch size				
	β	SE (β)	χ^2	d.f.	P
First broods					
Intercept	0.360	0.250			
Experimental density treatment	0.093	0.276	0.113	1	0.737
Location	1.256	0.263	14.276	1	<0.001
Year			33.673	10	<0.001
Experimental density treatment \times year			10.431	10	0.403
Location \times year			22.959	10	0.011
Random effects					
σ^2_{plot}	0.018	0.015	1.558	1	0.212
σ^2_{female}	0.347	0.041	71.852	1	<0.001
$\sigma^2_{\text{within female}}$	0.441	0.031	198.684	1	<0.001
Second broods					
Intercept	0.775	0.444			
Experimental density treatment	-0.798	0.488	2.668	1	0.102
Location	-0.426	0.468	0.828	1	0.363
Year			16.994	10	0.074
Experimental density treatment \times year			12.043	10	0.282
Location \times year			13.501	10	0.197
Random effects					
σ^2_{plot}	0.069	0.053	0.712	1	0.399
σ^2_{female}	0.123	0.116	1.124	1	0.289
$\sigma^2_{\text{within female}}$	0.663	0.123	29.157	1	<0.001

Table 2. Model summary examining the probability of having a second brood ($n = 1119$ females) in relation to experimental density treatments, location (north or south) and year. Significant values are shown in bold

Parameter	Probability of having a second brood				
	β	SE (β)	χ^2	d.f.	P
Intercept	-0.361	0.378			
Experimental density treatment	-0.504	0.323	2.433	1	0.119
Location	0.149	0.360	1.350	1	0.245
Year			131.165	10	<0.001
Random effects					
σ^2_{plot}	0.087	0.092	0.892	1	0.345
σ^2_{female}	1.436	0.330	18.888	1	<0.001
$\sigma^2_{\text{within female}}$	-	-	-	-	-

experimental treatment and location correlated significantly and negatively with annual density of first brood breeders. This correlation was significant for both low- and high-density plots (all: $F_{1,11} = 5.818$, $P = 0.039$; low-density plots: $F_{1,11} = 6.020$, $P = 0.036$; high-density plots: $F_{1,11} = 5.777$, $P = 0.040$).

NESTLING GROWTH

Nestlings from larger clutches grew less than from smaller clutches, and none of the growth parameters differed between experimental density treatments (Table 3, Fig. 4a). None of the nestling growth parameters was related to the location

(north/south). Wing length, however, varied significantly with year. For mass, the interactions experimental density treatments \times year and location \times year were significant (Table 3). The estimates of nestling growth parameters controlled for experimental density treatments, location, clutch size and the year interactions did not decrease significantly with annual density apart from the wing length in low-density plots (mass: all: $F_{1,10} = 1.67$, $P = 0.232$; low-density plots: $F_{1,10} = 1.31$, $P = 0.286$; high-density plots: $F_{1,10} = 1.37$, $P = 0.275$; Fig. 4b; wing length: all: $F_{1,10} = 3.157$, $P = 0.113$; low-density plots: $F_{1,10} = 9.725$, $P = 0.014$; high-density plots: $F_{1,10} = 2.186$, $P = 0.177$; tarsus: all: $F_{1,10} = 0.659$, $P = 0.440$; low-density plots: $F_{1,10} = 0.953$, $P = 0.357$; high-density plots: $F_{1,10} = 0.295$,

Table 3. Model summary examining growth parameters (mass, wing length and tarsus) of day 14 great tit nestlings in relation to experimental density treatment, location (north or south), nest clutch size, year, experimental density treatment \times year and location \times year ($n = 3859$ nestlings). The random effects reported are those from the mass model but the wing length and tarsus models gave similar values. Significant values are shown in bold

Parameter	β	SE (β)	χ^2	d.f.	<i>P</i>
Standardized mass					
Intercept	0.144	0.244			
Experimental density treatment	0.225	0.293	0.589	1	0.443
Location	0.343	0.321	1.137	1	0.286
Centred clutch size	-0.113	0.021	30.257	1	<0.001
Year			7.031	9	0.634
Experimental density treatment \times year			20.110	9	0.017
Location \times year			17.142	9	0.046
Standardized wing length					
Intercept	0.906	0.213			
Experimental density treatment	0.008	0.245	0.001	1	0.975
Location	0.145	0.276	0.274	1	0.601
Centred clutch size	-0.042	0.020	4.290	1	0.038
Year			37.884	9	<0.001
Experimental density treatment \times year			16.627	9	0.055
Location \times year			7.255	9	0.611
Standardized tarsus length					
Intercept	0.233	0.207			
Experimental density treatment	0.049	0.242	0.041	1	0.839
Location	-0.075	0.269	0.078	1	0.780
Centred clutch size	-0.057	0.019	8.892	1	0.003
Year			10.351	9	0.323
Experimental density treatment \times year			15.450	9	0.079
Location \times year			17.781	9	0.072
Random effects					
σ^2_{plot}	0.064	0.039	2.647	1	0.104
σ^2_{nest}	0.482	0.034	202.202	1	<0.001
$\sigma^2_{\text{individual}}$	0.369	0.009	1677.330	1	<0.001

$P = 0.601$). For nestling mass, however, the pattern changed when we analysed the data without the year 2001 when the mean nestling mass was much lower. Without 2001, nestling mass significantly decreased with an increase of annual density of the first brood breeders (Fig. 4b), but not when looking at low- and high-density plots separately (all: $F_{1,10} = 5.78$, $P = 0.047$; low-density plots: $F_{1,10} = 1.12$, $P = 0.325$; high-density plots: $F_{1,10} = 4.93$, $P = 0.062$).

WITHIN FEMALES

Individual females reduced their clutch size when annual D increased between breeding attempts, consistent with the 'individual adjustment' hypothesis (Table 4). This effect was nonlinear and significant also after correcting for age (Table 4). Females seemed to lay larger clutches in their 'middle' age, consistently with senescence pattern known in this species but the effect remained far from significant. The mean lifetime density experienced by a female did not explain the variation in individual clutch size.

Discussion

In great tits, the negative correlation between annual mean clutch size and annual breeding density is ubiquitous (Both

Table 4. Model summary examining clutch size variation within individual female in relation to the annual breeding density and its quadratic term, the mean individual lifetime density, the age of the female and its quadratic term. Only first broods are analysed ($n = 962$ females). Significant values are shown in bold

Parameter	Standardized clutch size				
	β	SE (β)	χ^2	d.f.	<i>P</i>
Intercept	-0.300	0.137			
Annual breeding density	-4.453	1.130	15.516	1	<0.001
Annual breeding density ²	35.794	11.92	9.019	1	0.003
Mean lifetime density	-0.034	1.307	0.001	1	0.975
Age	0.179	0.137	1.693	1	0.193
Age ²	-0.025	0.030	0.698	1	0.407
Random effects					
σ^2_{female}	0.457	0.053	74.777	1	<0.001
$\sigma^2_{\text{within female}}$	0.475	0.038	154.495	1	<0.001

2000). Breeding densities for our population at the Lauwersmeer are within the density range where density dependence of clutch size occurred in other populations, and indeed our non-experimental results confirm the general pattern (Both 1998a, Fig. 5). There is also some evidence that nestling mass (but not size) related negatively to annual breeding density.

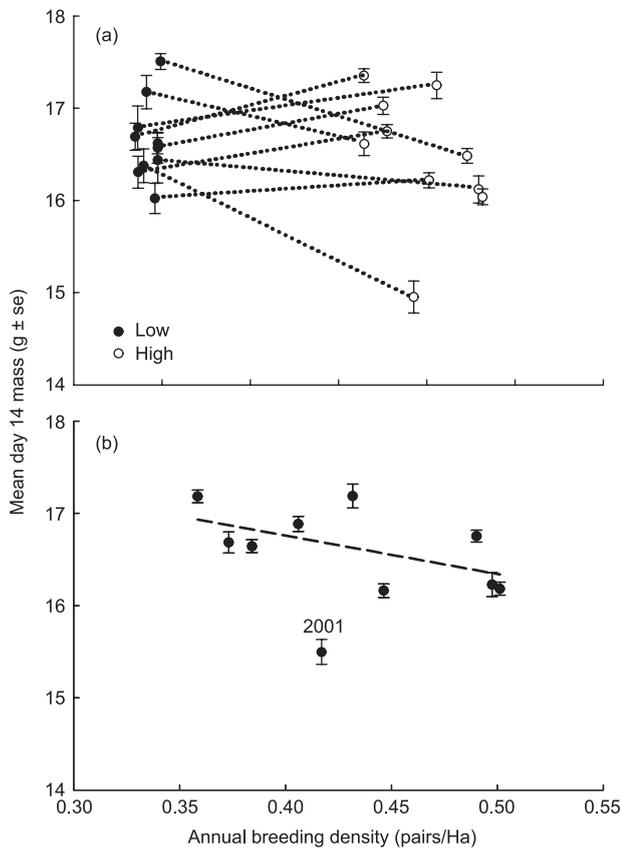


Fig. 4. (a) Within-year relationship between the mean population offspring mass at day 14 (\pm SE) and the local breeding density treatments (low density: closed circles; high density: open circles). (b) Relationship between the mean population offspring mass at day 14 (\pm SE) and the annual breeding density over the years. The dashed regression line in panel b represents the relationship between mass at day 14 mass and annual breeding density excluding the year 2001 from the analysis.

The decline of the population clutch size with annual breeding density was mainly due to changes occurring within individuals, and not by selective (dis)appearance of phenotypes. Individual females reduced their clutch size in response to an increase in annual breeding density, consistent with the individual adjustment hypothesis (Both 1998a). With regard to the association between breeding parameters other than first brood clutch size and annual breeding density, we found that the proportion of second clutches was higher in low-density years. The size of the second clutches positively correlated with the density of late breeders. A decreased competition for resources in low-density years may have stimulated the birds to produce more second clutches with a larger number of eggs.

Despite this congruence of the non-experimental data with previous descriptive studies, and in contrast to a previous density experiment (Both 1998b), we did not find a causal relation between local breeding densities and breeding parameters. The discrepancy between the absence of a density effect within years and the presence of a density effect between

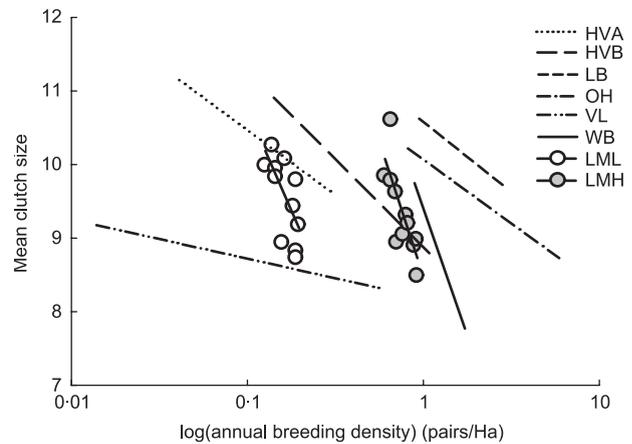


Fig. 5. Relationship between the mean population clutch size of six great tit populations in the Netherlands and their natural annual breeding densities (HVA: Hoge Veluwe A; HVB: Hoge Veluwe B; LB: Liesbos; OH: Oosterhout; VL: Vlieland; WB: Warnsborn; LML: Lauwersmeer low-density plots, white dots; LMH: Lauwersmeer high-density plots, grey dots). For more details see Both 1998a.

years is central to this paper and will be examined in detail after discussing the limitations of our experimental set-up.

LIMITATIONS OF THE EXPERIMENTAL SET-UP

We did not alter the experimental treatments of the plots between years. We may therefore have confounded experimental with natural variation. This was not the case however because clutch sizes before the experiment did not covary with later density treatment.

Manipulation of nest-box density may affect the competition for nesting sites and thereby the 'quality' of the birds that settle in them. If true, we would expect differences in bird 'quality' between the density treatments. For instance, in low-density plots, a high level of competition for nest boxes with a high level of antagonistic interactions might only allow 'high quality' birds to settle with an expected *increased* breeding performance. Alternatively, high competition might lead to negative effects on the breeding birds and hence to a *reduced* breeding performance. Low-density plots might also be unattractive (Fletcher 2007) resulting in settlement of 'low quality' birds (i.e. with expected low breeding output). We found that wing length, tarsus length and mass of breeding parents did not differ between the low- and high-density plots (corrected for year, analyses not shown here). Since we did not find differences in nestling mass nor in parental traits between the treatments, we judge these explanations unlikely and assume that the quality of the birds was equal between the treatments.

INTERPRETATION OF THE CLUTCH SIZE VARIATION

Within year, we did not find any effect of local densities on breeding output. This absence of a response to our density manipulation may relate to an overabundant food supply

during the nestling phase relative to the breeding density. However, judging from the mean nestling mass in our population this was not the case, especially in the later years, because it was comparable to the Hoge Veluwe population (Both *et al.* 2000). Both clutch size and breeding density in the Lauwersmeer area were also comparable to the other Dutch populations (Fig. 5). This indicates that the ecological circumstances of the Lauwersmeer must have differed from the other populations. Food during the nestling phase in the Lauwersmeer did not generate local competition.

Over the years, mean clutch size and the probability of producing a second clutch decreased while annual breeding density increased. Because annual breeding density and year were significantly correlated, any parameter changing over time may have caused the negative relation between clutch size and annual breeding density. We separately discuss the role of factors other than breeding density and annual breeding density to explain the annual clutch size variation in our population.

Hypothesis 1: environmental effects other than annual breeding density

Factors other than annual breeding density may have caused the observed decline in the clutch size and the occurrence of second clutches in our population over time. The decrease of nestling mass with annual breeding density (when 2001 was excluded) but not with local density might indicate that environmental conditions during the nestling phase have changed over time. Intraspecific variation in nestling growth often relates to environmental factors (e.g. Richner, Oppliger & Christe 1993; Keller & van Noordwijk 1994; Thessing 2000; Verboven, Tinbergen & Verhulst 2001). Our study was carried out in a young mixed deciduous forest reclaimed from the Wadden Sea in 1968 and planted 20 years before the start of our research. Consequently, the increase in breeding density over the years has coincided with the maturation of the forest and birds might thus have reacted to cues related to this maturation (e.g. change of food type) by decreasing their clutch size. Alternatively, other habitat properties (e.g. salinity of water or spring temperature) may have changed over time causing the decline in population clutch size. Detailed quantification and experimental manipulation of habitat parameters is needed if we want to judge causation because, as it is, any factor changing gradually over time may potentially be involved.

Hypothesis 2: density dependence at a landscape scale

The decrease of the population clutch size, of nestling mass and of the occurrence of second broods with an increase of annual population density between years may have resulted from a density-dependent process (e.g. review in Both 2000). Yet the fact that we did not find any effects of local densities on reproductive traits during the nestling phase contrasts with the experiment conducted by Kluijver in the same species (Liesbosch population; Both 1998b). In that experiment,

both clutch size and nestling growth were lower in the high-density treatment, suggesting that these reproductive parameters were affected by local competition. Moreover, Both *et al.* (2000) found that in the Hoge Veluwe population, the brood size maximizing fitness as estimated from brood size experiments differed with annual breeding density. They suggested that optimal density-dependent clutch size was mostly affected by the effect of density during the nestling stage.

The absence of an experimental effect on clutch size in our study implies that the density dependence of clutch size between years was not caused by local competition during the reproductive phase (when parents use restricted areas). The fact that selection on brood size was stabilizing in the Hoge Veluwe population (Tinbergen & Daan 1990) whereas it was positive in the Lauwersmeer population (Tinbergen & Sanz 2004; Tinbergen 2005) suggests that brood size was primarily limited by the amount of food parents can bring to their chicks in the Hoge Veluwe (Tinbergen & Dietz 1994) but not in the Lauwersmeer. The negative fitness effect of clutch enlargement in the Lauwersmeer (de Heij, van den Hout & Tinbergen 2006) also suggests that the parents were limited in the incubation phase rather than in the nestling phase in this population. Yet in this study, no effect of experimental local densities was found on clutch size, which we would expect if female condition determined by local competition during incubation would affect the fitness cost and benefits related to clutch size. We feel that this is an important result because it led us to the idea that competition acting at a landscape scale rather than a local scale could be involved.

To understand how a discrepancy between the effects of density within and between years on clutch size can arise, we depict in Fig. 6 the relationship between population clutch size and local density under variable competition levels at the landscape scale. If competition at the landscape scale is constant, independent of annual density (only one dotted line exists, Fig. 6), population clutch size would decrease with local density alone. As a consequence, within-year density variation and between-year density variation would yield similar patterns. In contrast, if competition at a landscape scale would increase with annual density (represented by different dotted curves relating clutch size to local density, Fig. 6), a discrepancy between the effects of local density (within years) and annual density (between years) would appear and may become apparent especially at the lower densities. The detection of density dependence may then depend on the range of densities studied. This is consistent with our findings.

In the Lauwersmeer population, competition at a landscape scale before or after the nestling phase may thus affect the clutch size-related fitness curve either via a parental or via an offspring component. In winter or early spring, food availability is low and birds can use large foraging areas leaving scope for competition at a large scale. Because great tit densities vary in parallel over large geographical areas according to large-scale variation in winter food abundance (Perrins 1965; Perdeck, Visser & Van Balen 2000; Saether *et al.* 2007), these birds will

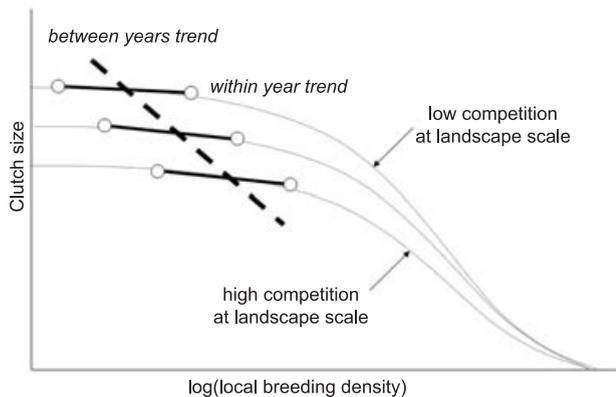


Fig. 6. Theoretical graph representing the relationship between optimal clutch size (i.e. associated with the highest fitness) and local breeding densities during the nestling phase under three different levels of competition at a landscape scale (dotted lines). At low densities, clutch size hardly varies with local density (our within-year effect, solid lines). There is no local competition for food. However, when density increases at a landscape scale, optimal clutch size decreases (dashed line) because of an increase in competition at a larger scale (in our data the between-year effect, dashed line). Variation in the strength of competition at different scales may thus explain a discrepancy between the effects of local density (within years) and annual density (between years) on observed clutch size especially at low local densities.

encounter similar densities in winter or early spring. If these densities affect female condition in early spring, and female condition in spring affects the clutch size related fitness curve, this may explain why females adjust their clutch size more to landscape density than to local density. Furthermore, when space is limited, the acquisition of a territory can be an important factor for population regulation and individual fitness because excluded individuals may not participate in reproduction (Begon, Harper & Townsend 1990). When annual breeding density increases, competition for space reduces the recruitment chance of juveniles that are subdominant to older birds (Sandell & Smith 1991). Therefore, it might pay for parents to adjust their clutch size and the number of broods they produce to the overall annual breeding density, in anticipation of the level of competition that juveniles will experience during settling. In this way, they may produce fewer but more competitive offspring that are more likely to recruit. Because natal dispersal can be considerable (Greenwood & Harvey 1982), this effect is likely to play out at a landscape scale. Such effects could provide the selective density dependent force that is consistent with our findings.

Conclusions

We did not find an experimental effect of local breeding density on reproductive parameters of great tits, whereas others did (Kluyver 1951; Dhondt *et al.* 1992; Both 1998b). However, clutch size of first broods, the proportion of second broods, and possibly also fledgling mass, all declined with increasing annual breeding density. If annual breeding density was causing the decline in clutch size of first broods

and the proportion of second broods in our population, in the absence of local competition we conclude that competition must have taken place at a landscape scale before or during egg laying or after fledging. Competition at a landscape scale is likely to be a general phenomenon acting simultaneously with competition at a local scale, but its relative importance would depend on ecological settings.

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References

- Arcese, P. & Smith, J.N.M. (1988) Effects of population density and supplemental food on reproduction in Song Sparrows. *Journal of Animal Ecology*, **57**, 119–136.
- Begon, M., Harper, J.L. & Townsend, C.R. (1990) *Ecology: Individuals, Populations and Communities*. Blackwell, Oxford, UK.
- Both, C. (1998a) Density dependence of clutch size: habitat heterogeneity or individual adjustment? *Journal of Animal Ecology*, **67**, 659–666.
- Both, C. (1998b) Experimental evidence for density dependence of reproduction in great tits. *Journal of Animal Ecology*, **67**, 667–674.
- Both, C. (2000) Density dependence of avian clutch size in resident and migrant species: is there a constraint on the predictability of competitor density? *Journal of Avian Biology*, **31**, 412–417.
- Both, C. & Visser, M.E. (2000) Breeding territory size affects fitness: an experimental study on competition at the individual level. *Journal of Animal Ecology*, **69**, 1021–1030.
- Both, C., Tinbergen, J.M. & Van Noordwijk, A.J. (1998) Offspring fitness and individual optimization of clutch size. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 2303–2307.
- Both, C., Tinbergen, J.M. & Visser, M.E. (2000) Adaptive density dependence of avian clutch size. *Ecology*, **81**, 3391–3403.
- de Heij, M.E., van den Hout, P.J. & Tinbergen, J.M. (2006) Fitness cost of incubation in great tits (*Parus major*) is related to clutch size. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2353–2361.
- Dhondt, A.A., Kempenaers, B. & Adriaenssens, F. (1992) Density-dependent clutch size caused by habitat heterogeneity. *Journal of Animal Ecology*, **61**, 643–648.
- Drent, P.J. (1984) Mortality and dispersal in summer and its consequences for the density of great tits *Parus major* at the onset of autumn. *Ardea*, **72**, 127–162.
- Fletcher, R.J. (2007) Species interactions and population density mediate the use of social cues for habitat selection. *Journal of Animal Ecology*, **76**, 598–606.
- Greenwood, P.J. & Harvey, P.H. (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13**, 1–21.
- Keller, L.F. & van Noordwijk, A.J. (1994) Effects of local environmental conditions on nestling growth in the Great Tit *Parus major* L. *Ardea*, **82**, 349–362.
- Kluyver, H.N. (1951) The population ecology of the Great Tit, *Parus m. major* L. *Ardea*, **39**, 1–135.
- Krebs, J.R. (1970) Territory and breeding density in Great Tit, *Parus major* L. *Ecology*, **52**, 2–22.
- Lack, D. (1966) *Population Studies of Birds*. Oxford University Press, Oxford, UK.
- Marra, P.P. & Holmes, R.T. (1997) Avian removal experiments: do they test for habitat saturation or female availability? *Ecology*, **78**, 947–952.
- Murdoch, W.W. (1994) Population regulation in theory and practice – the Robert H. MacArthur award lecture. *Ecology*, **75**, 271–287.
- Nager, R.G., Ruegger, C. & van Noordwijk, A.J. (1997) Nutrient or energy limitation on egg formation: a feeding experiment in great tits. *Journal of Animal Ecology*, **66**, 495–507.
- Newton, I. (1998) *Population Limitation in Birds*. Academic Press, London.
- Perdeck, A.C., Visser, M.E. & Van Balen, J.H. (2000) Great tit *Parus major* survival, and the beech-crop cycle. *Ardea*, **88**, 99–108.

- Perrins, C.M. (1965) Population fluctuations and clutch size in the great tit, *Parus major* L. *Journal of Animal Ecology*, **34**, 601–647.
- Pettifor, R.A., Perrins, C.M. & McCleery, R.H. (1988) Individual optimization of clutch size in great tits. *Nature*, **336**, 160–162.
- Pettifor, R.A., Perrins, C.M. & McCleery, R. (2001) The individual optimization of fitness: variation in reproductive output, including clutch size, mean nestling mass and offspring recruitment, in manipulated broods of great tits *Parus major*. *Journal of Animal Ecology*, **70**, 62–79.
- Rasbash, J., Steele, F., Browne, W. & Prosser, B. (2004) *A User's Guide to MLWIN*. Institute of Education, London.
- Richner, H., Oppliger, A. & Christe, P. (1993) Effect of an ectoparasite on reproduction in great tits. *Journal of Animal Ecology*, **62**, 703–710.
- Saether, B.E., Engen, S., Grotan, V., Fiedler, W., Matthysen, E., Visser, M.E., Wright, J., Moller, A.P., Adriaensen, F., Van Balen, H., Balmer, D., Mainwaring, M.C., McCleery, R.H., Pampus, M. & Winkel, W. (2007) The extended Moran effect and large-scale synchronous fluctuations in the size of great tit and blue tit populations. *Journal of Animal Ecology*, **76**, 315–325.
- Sandell, M. & Smith, H.G. (1991) Dominance, prior occupancy, and winter residency in the great tit (*Parus major*). *Behavioral Ecology and Sociobiology*, **29**, 147–152.
- Sillett, T.S., Rodenhouse, N.L. & Holmes, R.T. (2004) Experimentally reducing neighbor density affects reproduction and behavior of a migratory songbird. *Ecology*, **85**, 2467–2477.
- Sinclair, A.R.E. (1989) Population regulation in animals. *Ecological Concepts* (ed. J.M. Cherrett), pp. 197–241. Blackwell, Oxford.
- Soler, M. & Soler, J.J. (1996) Effects of experimental food provisioning on reproduction in the Jackdaw *Corvus monedula*, a semi-colonial species. *Ibis*, **138**, 377–383.
- Thessing, A. (2000) Growth and final size of willow tit *Parus montanus* under different environmental conditions. *Ardea*, **88**, 215–224.
- Tinbergen, J.M. (2005) Biased estimates of fitness consequences of brood size manipulation through correlated effects on natal dispersal. *Journal of Animal Ecology*, **74**, 1112–1120.
- Tinbergen, J.M. & Both, C. (1999) Is clutch size individually optimized? *Behavioral Ecology*, **10**, 504–509.
- Tinbergen, J.M. & Daan, S. (1990) Family planning in the great tit (*Parus major*) – optimal clutch size as integration of parents and offspring fitness. *Behaviour*, **114**, 161–190.
- Tinbergen, J.M. & Dietz, M.W. (1994) Parental energy expenditure during brood rearing in the great tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. *Functional Ecology*, **8**, 563–572.
- Tinbergen, J.M. & Sanz, J. (2004) Strong evidence for selection for larger brood size in a great tit population. *Behavioral Ecology*, **15**, 525–533.
- Tomba, F.S. (1967) Reproductive success in relation to breeding density in pied flycatchers, *Ficedula hypoleuca*. *Acta Zoologica Fennica*, **118**, 1–28.
- Török, J. & Toth, L. (1988) Density dependence in reproduction of the collared flycatcher (*Ficedula albicollis*) at high population levels. *Journal of Animal Ecology*, **57**, 251–258.
- Verboven, N., Tinbergen, J.M. & Verhulst, S. (2001) Food, reproductive success and multiple breeding in the great tit *Parus major*. *Ardea*, **89**, 387–406.
- Verhulst, S. (1992) Effects of density, beech crop and winter feeding on survival of juvenile great tits: an analysis of Kluver's removal experiment. *Ardea*, **80**, 285–292.
- Watson, A. & Jenkins, D. (1968) Experiments on population control by territorial behaviour in red grouse. *Journal of Animal Ecology*, **37**, 595–&.
- Wilkin, T.A., Garant, D., Gosler, A.G. & Sheldon, B.C. (2006) Density effects on life-history traits in a wild population of the great tit *Parus major*: analyses of long-term data with GIS techniques. *Journal of Animal Ecology*, **75**, 604–615.

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