Family planning under social competition

Fokkema, Rienk

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Is parental competitive ability in winter negatively affected by previous springs’ family size?

Rienk. W. Fokkema
Richard Ubels
Joost M. Tinbergen

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Reproductive behaviour cannot be understood without taking the local level of competition into account. Experimental work in great tits (*Parus major*) showed that 1) a survival cost of reproduction was paid in environments with high levels of competition during the winter period and 2) that experimentally manipulated family size negatively affected the ability of parents to compete for preferred breeding boxes in the next spring. The fact that survival was affected in winter suggests that the competitive ability of parents in winter may also be affected by previous reproductive effort. In this study we aim to investigate whether 1) such carry-over effects of family size on the ability of parents to compete for resources in the winter period occurred and 2) whether this could explain the occurrence of a survival cost of reproduction under increased competition. During two study years, we manipulated the size of in total 168 great tit broods. Next, in winter we induced competition among the parents by drastically reducing the availability of roosting boxes in their local environment for one week. Contrary to our expectation, we found no negative effect of family size manipulation on the probability of parents to obtain a roosting box. In line with previous work we did find that a survival cost of reproduction was paid only in plots in which competition for roosting boxes was shortly increased. Our findings thus add to the scarce experimental evidence that survival cost of reproduction are paid under higher levels of local competition but this could not be linked to a reduced competitive ability of parents in winter.
INTRODUCTION

Competition within the social environment of a parent may be an important selective force on its reproductive behaviour (Svensson and Sheldon 1998; Nicolaus et al. 2012a; Wilson 2014). Life history theory (Schaffer and Rosenzweig 1977; Roff 1992; Stearns 1992) identifies two fundamental trade-offs that determine individual reproductive decisions: 1) the trade-off between quality and quantity of offspring (Lack 1947) and 2) the trade-off between current- and future reproduction (Williams 1966). Here we focus on the mechanism behind the parental cost of reproduction, an important component of the second trade-off.

The trade-off between current and future reproduction implies that if a parent increases its investment into current reproduction this leads to physiological costs for the parent, in turn these physiological costs are expected to lead to fitness costs of reproduction (i.e. survival or fecundity costs; Williams 1966; for review see: Speakman 2008). The actual empirical evidence, especially for a survival cost of reproduction, has proved mixed however (avian: Linden and Møller 1989; Dijkstra et al. 1990; Stearns 1992; Golet et al. 1998; Parejo and Danchin 2006; Santos and Nakagawa 2012; mammals: Stearns 1992; Hamel et al. 2010), in some populations a survival cost of reproduction has been detected, but not in others.

One reason for this discrepancy may be that a survival cost of reproduction may only occur when competition in the social environment is high (Oksanen et al. 2007; Nicolaus et al. 2012a). In experimental work by Nicolaus et al. (2012) on great tits (Parus major) it was shown that parents, during the winter period, paid a survival cost of reproduction in areas with increased competition, but not in areas with low competition. The authors hypothesized that family size negatively affected the competitive ability of parents and under high competition this led to a survival cost of reproduction. If so, such carry over effects (Harrison et al. 2011; O’Connor et al. 2014) could provide a causal explanation for the general pattern that parents reduce their reproductive investment at higher population density and presumably competition (e.g. avian: Kluijver 1951; Perrins 1965; Both et al. 2000; Nicolaus et al. 2013; mammals: Morris 1989; Koskela et al. 1999; Bonenfant et al. 2009).

In a recent study we found first evidence that indeed family size negatively affects the competitive ability of parents in the next spring (Fokkema et al. 2016). Consistently over two study years, experimentally manipulated family size had a negative effect on the ability of great tit (Parus major) parents to claim a high quality breeding box the following spring. The result of Nicolaus et al. (2012) that a survival cost of reproduction was paid only in environments with high competition in winter suggests that similar negative effects of family size on the ability of parents to compete for resources in winter may exist. Here we aim to directly test if 1) such carry-over effects of family size on the ability of parents to compete for resources in the winter period occurred and 2) whether this could explain the occurrence of a survival cost of reproduction under increased competition in winter (as observed by Nicolaus et al. 2012a). By doing this we gain insight when in the lifecycle of a parent competitive ability is affected by earlier reproductive effort. This
knowledge is vital to predict selection on reproductive investment under local competition.

One important resource for which competition in winter may occur is the availability of roosting boxes. Roosting in a nest box, as opposed to roosting outside, may enhance winter survival of birds by decreasing thermoregulatory costs and the risk of predation (Drent 1987; Mainwaring 2011; Atema et al. 2016). We expected that if the availability of roosting boxes was limited, experimentally manipulated family size would negatively affect the ability of parents to claim a roosting box in the subsequent winter. This in turn could result in a survival cost of reproduction for the parents involved.

To test our expectation, we experimentally manipulated family size during two study years. Subsequently, at midwinter, we induced competition among the manipulated great tit parents for roosting boxes for a short period by strongly reducing the availability of roosting boxes in half of the study area. We next quantified the effect of family size manipulation on the ability of parents to claim a roosting box and on the apparent survival of parents in relation to the increased competition for roosting boxes.

**METHODS**

**Study area and study population**

We studied a nest box breeding great tit population in the Lauwersmeer area in the north of the Netherlands (coordinates: 53°23’N, 6°14’E). The area of approximately 24 km² was planted in 1969 resulting in a relatively young (approx. 40 years old) mainly deciduous forest interspersed with grassy areas. The nest box population comprised 12 non adjacent plots with 50 boxes each, resulting in 600 nest boxes in total (Nicolaus et al. 2009).

Ethical permission for this study was given by the Animal Experiments Committee (DEC project: 5548F).

**Breeding season**

*Monitoring egg laying and breeding*

We checked all 600 nest boxes every week during the breeding season of 2012, 2013 and 2014. If eggs were encountered in a nest box we calculated the first egg laying date, assuming that one egg was laid a day. Next, as soon as we detected that clutches were incubated we calculated an expected hatching date. This calculation was done on the basis of the first egg laying date and the clutch size, assuming that breeding started directly after the last egg was laid, and that the incubation period lasted 12 days (e.g. de Heij et al. 2006). We checked all incubated nests daily 1–2 days before the expected hatching date (day 0) and this continued until the first egg hatched.

*Family size manipulation*

Five days after hatching, we visited the nest again, recorded the number of nestlings and weighed the entire brood (mass ± 0.1 g). Using these data (see below) we manipulated the family sizes the next day (day 6). Family sizes were manipulated as follows. When the
nestlings were 6 days old we matched a set of three nests with a similar hatching date according to the number of nestlings, clutch size and brood weight (hereafter called ‘trio’; for analysis purposes each trio was assigned a number to correct for non-independence, see: ‘random effects included’). Within the trio we randomly assigned nest treatment and the nestlings to exchange: one family was enlarged, one family was reduced and one family was kept as a control. We exchanged three nestlings in most trios (2012: N = 28 trios, 2013: N = 21 trios; both years: average number of nestlings pre exchange: Reduced: 8.5, Control: 8.3, Enlarged: 8.4; post exchange: R: 5.5, C: 8.3, E: 11.4). In some cases however we exchanged two nestlings (2012: N = 3 trios, 2013: N = 4 trios; both years: average number of nestlings pre exchange: R: 6.4, C: 6.6, E: 6.6; post exchange: R: 4.4, C: 6.6, E: 8.6). We did this to prevent brood desertion when, after reduction, the family size would be less than 5 nestlings (Verboven and Tinbergen 2002). To ensure that broods were disturbed to a similar extent and that the fraction of own nestlings relative to the total number of offspring remained approximately the same, we also exchanged four nestlings of the control brood, two with two nestlings of the reduced brood and two with two nestlings of the enlarged brood (for further details see: de Jong et al. 2014; Fokkema et al. 2016).

We subsequently measured whether the family size manipulation (hereafter termed ‘FS manipulation’) successfully increased parental feeding effort judged by three components, the number of visits made by each parent per day, the gain in weight of the brood after FS manipulation and the number of fledglings produced. The number of visits made by each parent and the number of fledglings produced successfully increased with FS manipulation, no such effect was found on the gain in weight of the manipulated broods (see: appendix S1).

Identification of parents and providing RFID transponder rings
During the nest box checks we visually identified the incubating female, if possible, when she was sitting tight on the eggs, on the basis of a previously applied unique combination of colour rings to her legs. The day after FS manipulation (nestlings 7 days old), we additionally caught both parents (also the previously identified females during incubation) using spring traps inside the nest box. If parents could not be caught the day after FS manipulation a second attempt was made two days later. When caught, parents were identified based on the existing identification rings (aluminium ring with unique inscription and three plastic colour rings). If not yet ringed, we provided parents with identification rings. In 2013, we altered the colour ring scheme and 146 caught parents which raised a manipulated brood were provided with an RFID transponder ring (type: EM4102 bird PIT tag 2.6mm, manufactured by: IB technology, Eccel Technology Limited; each bird was provided with a transponder ring, an aluminium ring and two plastic colour rings; see fig. 2.1). These transponder rings enabled us to measure the effects of FS manipulation on parental feeding effort during the breeding season (see: appendix S1) and identification while roosting in winter (see ‘occupation of the roosting boxes after competition was induced’). Some parents could not be caught at all, these were identified if possible, using binoculars (again based on existing colour rings).
Figure 2.1: A great tit (Parus major) with an RFID transponder ring (light blue). The transponder ring enabled measurements of the number of feeding visits made by parents in response to family size manipulation and identification without disturbance when roosting. On the legs of the bird additionally a colour ring and an aluminium ring were fitted to enable visual identification. Picture taken by: Richard Ubel.

Injuries due to transponders

Unexpectedly, 9% of the parents with a transponder ring in 2013 developed injuries after the breeding season (swollen leg, sometimes necrosis; injuries first detected in November) on the leg to which this ring was fitted (13 of the 146 parents with a transponder ring). We showed that the feeding effort of parents in 2013 did increase with FS manipulation (appendix S1) and that the manipulation groups did not differ in their probability to get injured ($\chi^2_{\text{d.f.}=NA} = 2.92, P = 0.25$, degrees of freedom could not be calculated, see ‘model selection’). Any effects of injuries due to the transponders on the competitive ability of parents or their local survival probability were thus not likely to differ between the FS manipulation groups. Injured birds were treated by removing their transponder ring. We additionally removed the plastic colour ring attached to the same leg as the transponder for birds with no injuries. The latter treatment did not prevent injuries altogether. In 2014, for a different experiment, 2 parents developed injuries (of the 184 parents provided with a single transponder ring this year). In general, the injuries did not seem to cause increased mortality and 4 of the 5 parents with serious injuries (necrotic legs) were even able to start a new brood the following season (overall: 10 of the 13 parents observed with injuries were able to start a brood in 2014 (77%), average local survival of parents in this period for 2010-2012: 43%).
Midwinter competition experiment

*Inducing competition for roosting boxes*

At the beginning of December in 2012 and 2013 (termed ‘midwinter’ hereafter) we spend two consecutive evenings checking all nest boxes in our study area for roosting birds (12 study plots; 50 boxes per plot). Two nights after this roost check, we induced competition in 6 of the 12 study plots in the area (termed ‘experimental plots’ hereafter; the uneven numbered plots in 2012 and the even numbered plots in 2013) and kept the remaining six plots as controls (fig. 2.2).

We induced competition in the experimental plots by, at daytime, plugging up the entrance hole of all 50 boxes available per study plot and redistributing 10 new roosting boxes at new locations in the plot. We chose to reduce the number of boxes from 50 to 10 to induce competition in all study plots as the number of sleeping great tits per plot differed markedly (between 8 and 44 great tits roosted per study plot in earlier roost checks at midwinter in 2010 and 2011). We chose the locations of the new boxes relative

![Diagram](image)

**Figure 2.2:** Time line of the experiments relative to the annual cycle. In 2012 and 2013 family size was manipulated when the nestlings were 6 days old (second column, grey lines with triangles pointing right). In the subsequent winter the number of roosting boxes was reduced by 80 percent in half of the study area (‘the experimental plots’; third column, right pointing triangles). The other half of the plots were kept as a control. One week later we restored the number of roosting boxes (left pointing triangles). We measured the local survival probability of parents during two periods (fourth column): A) from the breeding season until the time point competition for roosting boxes was induced and B) from the time point that competition was induced until the following breeding season. For further explanation see methods.
to a randomly assigned selection of 10 of the old nest box locations in the plot. We put up the new boxes 25 meters to the north-east of these locations. If this location was not suitable (e.g. no trees available) we placed the new box 25 meters to the south-east. Analysis showed that the FS manipulation groups did not differ in the distance from the box in which parents roosted before the experiment to the nearest available new box (linear model: $F_{(2, 63)} = 0.35$, $P = 0.70$, average distance to a new roosting box: 58 meters).

During the week of competition the average local temperatures were the following in 2012: $T_{	ext{min}} = 0.9^\circ\text{C}$, $T_{\text{max}} = 4.9^\circ\text{C}$ and 2013: $T_{\text{min}} = 3.4^\circ\text{C}$, $T_{\text{max}} = 7.5^\circ\text{C}$. In 2013 but not in 2012, throughout the study area we provided supplementary food at feeding stations for a different experiment. Using transponder readers we could identify which manipulated parents visited these feeding stations and how often. Based on this data, we found no evidence that parents of the manipulation groups used the supplementary food differentially (generalized linear model with quasi-Poisson error structure: $F_{(2, 23)} = 0.02$, $P = 0.98$, average number of visits to feeder: 237). These differences in winter conditions between study years were taken into account in our analyses by including study year and the interaction between family size manipulation and study year (see: ‘statistics’). Few natural cavities were likely available to the birds as alternative to the nest boxes as the forest is relatively young (Newton 1994); (see ‘study area and study population’).

**Occupation rate of the roosting boxes after competition was induced**

In 2013, spread over the week that competition for roosting boxes was induced, we performed two roosting checks in the experimental plots, to monitor box occupation over time (1–2 and 3–4 days after competition was induced). We used handheld readers (type: LID575-IS0; manufactured by Dorset identification b.v.) which could read the code emitted by the transponder of the parents through the bottom of the box (to minimize the disturbance of the roosting birds). Our data shows that parents found the new boxes soon after competition was induced (1–2 nights after roosting 70% of the manipulated parents ($N = 10$) observed in the final night check at the end of the experiment (see section below) were detected). We never observed that roost boxes had different owners during the experiment. Those parents that were detected multiple times in the roost checks during the experiment were observed to roost in the same box.

**Determining the winners of the competition for roosting boxes**

In both years, seven nights after competition for roosting boxes was induced, we performed a final roost check in the experimental- and the control plots to determine which manipulated parents were able to claim a roosting box (the winners). During this roost check, like in the roost check before the onset of the midwinter competition experiment, boxes were opened and birds were taken out and identified (both years combined: $N = 43$ parents observed roosting in the control plots of 50 parents observed before competition was induced; $N = 27$ parents observed roosting in the experimental plots of 66 parents observed before). None of the manipulated parents moved between plots during the experiment.
Parental fitness components
To assess whether a survival cost of reproduction was paid before- or after the time point that competition for roosting boxes was experimentally induced, we calculated 1) the local survival probability of both parents over the period from the breeding season (N = 323) until midwinter (N = 155) and 2) the local survival probability of both parents over the period from midwinter until the following breeding season (N = 46; fig. 2.2). For our measure of local survival of parents from the breeding season until midwinter, we deemed all parents observed roosting in the first night check as alive plus those later seen alive during the breeding season. Local survival after midwinter until the following breeding season, was determined based on recaptures during the breeding season (local survival in our study thus corresponds to apparent survival). Mark-recapture models were not used to estimate parental local survival because the adult detection probability if alive in the breeding season is high in this population (0.897, SE = 0.055 see: Tinbergen and Sanz 2004).

Statistics
We used R (version 3.2.3; R Core Team 2015) and the package ‘lme4’ (Bates et al. 2015) to create the mixed models. The effects of FS manipulation on the probability of parents to claim a roosting box and on parental local survival were analysed using generalized linear mixed effects models (GLMER) with a Binomial error structure.

Predictor variables included
Within all analyses, FS manipulation was included as a continuous variable because of our directional expectation (directional statistical tests, see: Fokkema et al. 2016, Knowles et al. 2009) and we allowed for non-linear effects by including a quadratic effect of FS manipulation. Next to this, we included three other predictor variables. We included the factors study year and sex of the parent in all analyses and the factor plot treatment (experimental- or control plots) in the analyses of the probability of parents to claim a roosting box and parental local survival after midwinter. All three above described factors were tested as main effect and in interaction with FS manipulation and FS manipulation2.

Random effects included
We included three random variables in all analyses: 1) ‘trio’ number, this factor was included to correct for non-independence of the matched trios of nests (see methods: ‘family size manipulation’), 2) brood ID, this identification number for the brood raised, was included to account for the fact that some parents had a shared history, and 3) individual ID, this identification number for the individual parent, was included as a factor to account for the fact that some individuals were observed during both experimental years (N = 21 individuals with repeated measurements within the analysis of local survival until the midwinter and N = 5 individuals with repeated measurements in the both the analysis of the probability of a parent to claim a roosting box and the probability of a parent to survive after midwinter).
Model selection
Our aim was to get the most accurate estimate of the effects of FS manipulation. We therefore tested which predictor variables, that did not significantly aid in estimating the effect of FS manipulation, could be eliminated. We first tested whether the interactions between FS manipulation$^2$ and all included predictor variables could be eliminated in order of significance. Next we eliminated the interactions between FS manipulation and the predictor variables if non-significant. Then FS manipulation$^2$ was removed if possible and finally all other predictor variables were removed in order of their significance. This backward elimination procedure was done on the basis of likelihood ratio tests. We kept the random effects in the models at all times during model selection as these were there to correct for non-independence in the dataset (as in Fokkema et al. 2016).

The chi-square goodness of fit test presented in the methods section ‘injuries due to transponders’ was done using simulated P-values, due to the low sample size. When using this method, the degrees of freedom cannot be given and are presented as NA (Hope 1968). Package ‘ggplot2’ (Wickham 2009) was used to construct the figures. We calculated the solid lines in figure 2.3 using the predict function of package ‘lme4’ on the basis of the selected models.

RESULTS

Family size manipulation and the local survival probability of parents until midwinter
We found no effects of FS manipulation on the local survival probability of parents until midwinter (FS manipulation: $\chi^2_{d.f.1} = 0.53, P = 0.47$; FS manipulation$^2$: $\chi^2_{d.f.1} = 0.001, P = 0.97$; average local survival probability of parents: Reduced: 0.51 (95% CI = 0.42, 0.61), Control: 0.47 (95% CI = 0.37, 0.56), Enlarged: 0.46 (95% CI = 0.37, 0.55), for the latter comparison we grouped the $-3/-2$ and the $+2/+3$ FS manipulations). We further found no indication that the direction of the effect of FS manipulation on parental local survival differed depending on the study year or sex of the parent or that the factors sex of the parent and study year independently played a role.

Midwinter experiment: competition for roosting boxes
Family size manipulation and the probability to occupy a roosting box before competition for roosting boxes was induced
Controlling for effects of study year and sex of the parent (lower probability of roosting boxes to be occupied in 2013: intercept: $-0.91 \pm 0.25$, $\beta = -0.60 \pm 0.31$, $\chi^2_{d.f.1} = 4.02$, $P < 0.05$; higher probability of males to occupy a roosting box: $\beta = 0.95 \pm 0.27$, $\chi^2_{d.f.1} = 13.58$, $P < 0.001$) we found no effect of FS manipulation on the probability to encounter a parent in a roosting box in the first roost check (FS manipulation: $\chi^2_{d.f.1} = 0.87, P = 0.35$; FS manipulation$^2$: $\chi^2_{d.f.1} = 0.003, P = 0.96$; average probability of parents to occupy a roosting box: Reduced: 0.39 (95% CI = 0.31, 0.49), Control: 0.35 (95% CI = 0.27, 0.45), Enlarged: 0.33 (95% CI = 0.25, 0.43), for the latter comparison we grouped the $-3/-2$ and
the +2/+3 FS manipulations). We further found no indication that the direction of the effect of FS manipulation on the probability to observe parents in a roosting box differed depending on the study year or sex of the parent.

*Fraction of roosting boxes occupied after competition for roosting boxes was induced*

Consistent with what we would expect if competition occurred, the fraction of roosting boxes occupied by great tits in the experimental plots significantly increased (see table 2.1 for absolute numbers of roosting boxes occupied; chi-square goodness of fit test: 2012: $\chi^2_{\text{d.f.1}} = 5.71, P < 0.05$; 2013: $\chi^2_{\text{d.f.1}} = 23.77, P < 0.001$). In 2012, in the control plots the fraction of occupied roosting boxes by great tits decreased slightly ($\chi^2_{\text{d.f.1}} = 6.18, P < 0.05$), while in 2013 it remained constant ($\chi^2_{\text{d.f.1}} = 1.33, P = 0.25$).

The fraction of roosting boxes occupied by the sub-dominant blue tit (*Cyani
ger caerul
erus*; the only species that makes use of roosting boxes in our study area besides the great tit) stayed constant in the experimental plots (2012: $\chi^2_{\text{d.f.1}} = 0.29, P = 0.59$; 2013: $\chi^2_{\text{d.f.1}} = 0.20, P = 0.65$) and in the control plots (2012: $\chi^2_{\text{d.f.1}} = 0.27, P = 0.61$; 2013: $\chi^2_{\text{d.f.1}} = 1.23, P = 0.27$). The number of empty boxes in the experimental plots decreased significantly (2012: $\chi^2_{\text{d.f.1}} = 4.21, P < 0.05$; 2013: $\chi^2_{\text{d.f.1}} = 21.34, P < 0.001$), while in the control plots the number of empty boxes increased in 2012 ($\chi^2_{\text{d.f.1}} = 5.61, P < 0.05$) and stayed constant in 2013 ($\chi^2_{\text{d.f.1}} = 3.54, P = 0.06$).

*Family size manipulation and the probability of parents to claim a scarce roosting box*

Against expectation, after competition for roosting boxes was induced, we found no effect of FS manipulation on the ability of parents to claim a roosting box (corrected for effects of sex and plot treatment: table 2.2; average probability to obtain a box: experimental plots: R: 0.38 (95% CI = 0.23, 0.56), C: 0.45 (95% CI = 0.26, 0.66), E: 0.47 (95% CI = 0.26, 0.69); control plots: R: 0.69 (95% CI = 0.42, 0.87), C: 0.88 (95% CI = 0.66, 0.97), E: 0.90

<table>
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</thead>
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<tr>
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<tr>
<td>Control plots</td>
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<td>300 boxes available</td>
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</tr>
<tr>
<td>2013</td>
<td>136</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 2.1: Overview of the number of roosting boxes occupied in both the control- and experimental plots by great and blue tits before and after competition for roosting boxes was induced in the experimental plots.
Table 2.2: Outcome of the generalized linear mixed effects model describing the effects of family size manipulation on the probability of parents to claim a roosting box (N = 116 parents). The probability to claim a roosting box was much lower in the experimental plots, but no effect of family size manipulation could be detected. The variance of the random effect trio was 8.3e⁻², the variance of the random effect brood id was 0 and the variance of the random effect individual id was 2.3e⁻⁹.

<table>
<thead>
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<th>Variable</th>
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<th>Δχ²</th>
<th>d.f.</th>
<th>P</th>
</tr>
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<tr>
<td>Male effect (relative to female)</td>
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<tr>
<td>Experimental plots (relative to control plots)</td>
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<td>&lt;0.001</td>
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Rejected terms:
- manipulation² x plot treatment (d.f.1), manipulation² x sex (d.f.1), manipulation² x year (d.f.1), manipulation x plot treatment (d.f.1), manipulation x sex (d.f.1), manipulation x year (d.f.1), manipulation² (d.f.1), manipulation (d.f.1), year (d.f.1)

(95% CI = 0.70, 0.97). We further found no evidence that the direction of the effect of FS manipulation on the probability of parents to claim a roosting box differed with plot treatment, year or sex.

Effects of family size manipulation on fitness components after midwinter

Controlled for effects of study year, we found that experimentally manipulated family size did have a consistent negative effect on the local survival probability of parents from midwinter until the breeding season in plots where we induced competition for roosting boxes, but not on the local survival probability of parents in control plots (fig. 2.3; table 2.3). In the control plots effects of FS manipulation seemed to work in the opposite direction. The effect of FS manipulation did not differ between study years or between the sexes. There further was no evidence for a non-linear effect of FS manipulation, nor for a difference between the sexes. Further analysis showed a trend that the survival cost of reproduction in the experimental plots was paid only within the group of parents that were able to claim a roosting box during the experiment (fig. 2.4; controlled for year effect; FS manipulation x claimed box: intercept: −7.18 ± 4.39, β = −1.45 ± 1.53, χ²_{d.f.1} = 2.82, P = 0.09).

There was no indication that the observed effects in the experimental plots of FS size manipulation on parental local survival from midwinter until the following breeding season were the consequence of selective dispersal rather than selective mortality. In both the control and the experimental plots there was no effect of FS manipulation on the distance moved between the roosting box in which a parent was observed at midwinter and the box it used for breeding the following spring (linear model: FS manipulation x plot treatment: F_{1, 41} = 0.31, P = 0.58, FS manipulation: F_{1,42} = 1.28, P = 0.26; controlled for effects of sex and year; average distance moved to breeding box: 84 meters).
Figure 2.3: The effect of family size manipulation on the local survival probability of parents from midwinter until the next breeding season. A survival cost of reproduction was only paid in the plots in which competition was induced. Black dots depict manipulations in which three nestlings were exchanged; grey dots depict manipulations in which two nestlings were exchanged. Sample size is indicated by symbol size and the numbers next to the 95% confidence intervals. The solid line depicts the predicted response calculated on the basis of the final selected model.

Table 2.3: Outcome of the generalized linear mixed effects model describing the effects of family size manipulation on the local survival probability of parents resident in the control and the experimental plots from midwinter to the following breeding season (N = 116 parents). The variance explained by the random effect trio was 1.18, the variance of the random effect brood id was 0 and the variance of the random effect ring number which coded for the individual was 7.67e−15.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate $\beta$ (±SE)</th>
<th>$\Delta \chi^2$</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−1.16 (0.55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family size manipulation</td>
<td>0.27 (0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family size manipulation x plot treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental plots (relative to control plots)</td>
<td>−0.55 (0.26)</td>
<td>4.80</td>
<td>1</td>
<td>&lt;0.05</td>
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<tr>
<td>Year</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2013 (relative to 2012)</td>
<td>3.34 (0.83)</td>
<td>25.15</td>
<td>1</td>
<td>&lt;0.001</td>
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<tr>
<td>Plot treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (relative to control)</td>
<td>−1.73 (0.66)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rejected terms: manipulation² x sex (d.f.1), manipulation² x year (d.f.1), manipulation² x plot treatment (d.f.1), manipulation x sex (d.f.1), manipulation x year (d.f.1), manipulation² (d.f.1), sex (d.f.1)
**Figure 2.4**: The effect of family size manipulation on the local survival probability of parents that did not and parents that did occupy a roosting box after competition for them was induced. The data suggest a survival cost of reproduction for those birds that did occupy a roosting box, in contrast to those that did not occupy a roosting box. Black dots depict manipulations in which three nestlings were exchanged; grey dots depict manipulations in which two nestlings were exchanged. Sample size is indicated by symbol size and the numbers next to the 95% confidence intervals. The solid line depicts the predicted response calculated on the basis of the final selected model.

**DISCUSSION**

The aim of this study was to test whether 1) family size affects the ability of great tit parents to compete for roosting sites in the winter period and 2) whether this could explain the occurrence of a survival cost of reproduction under increased competition in winter (as shown by Nicolaus et al. 2012a). Such a carry-over effect of family size on parental competitive ability and subsequent fitness (Harrison et al. 2011; O’Connor et al. 2014) could provide a causal explanation why selection would favour smaller families at higher population density (e.g. avian: Kluijver 1951; Perrins 1965; Both et al. 2000; Nicolaus et al. 2013; mammals: Morris 1989; Koskela et al. 1999; Bonenfant et al. 2009). After experimental reduction of the number of roosting boxes in winter we found that 1) prior family size manipulation did not affect the ability of great tit parents to claim one of the scarce roosting boxes, but 2) we did find that the short period of increased competition for roosting boxes resulted in a survival cost of reproduction, strengthening the scarce experimental evidence (Nicolaus et al. 2012a) that survival cost of reproduction depend on the competitive situation. Yet, the question how a survival cost of reproduction is paid under competition remains unsolved; it was not due to a reduced ability of parents to claim a scarce roosting box.
Family size and competitive ability
Here we explore two possible reasons why a negative effect of family size on the ability of parents to claim a roosting box was not apparent.

1) *Were the costs of reproduction already paid before midwinter?*
One potential reason for not finding a negative effect of family size on the competitive ability of parents is that parents already paid a survival and/or fecundity cost of reproduction in full before midwinter. This could happen because parents with different reproductive effort experienced differential survival till midwinter, but we found no evidence that this was the case. Alternatively it could happen because parents as a consequence of the manipulation of the size of their first brood differentially invested in late broods within the same breeding season. This was the case in 2013 (see appendix S1) but not in 2012. In 2013 parents in the different manipulation groups may thus have had a similar reproductive investment over the whole breeding season. Despite this we found no difference between the years 2012 and 2013 in the negative effect of family size manipulation on the local survival of parents after midwinter in the plots in which competition was induced. In line with previous studies (Nicolaus et al. 2012a; Fokkema et al. 2016), parents did thus not fully compensate for effects of family size manipulation in 2013 by foregoing a late brood within the same season. We thus have no evidence that costs of reproduction were already paid in full before mid-winter.

2) *Was there competition among parents for roosting boxes?*
It may be that, effects of family size on parental competitive ability were there, but that we could not detect them, because we did not successfully induce competition among the parents by reducing the availability of roosting boxes. It is well established, that roosting boxes are an important resource for great tits in winter to evade predation and reduce thermoregulatory costs (Drent 1987; Mainwaring 2011). Potentially, roosting boxes were less important in this respect in 2013 than in 2012, because in this year the local temperature in winter was higher and supplementary food was provided (for a different study). Consistent with this, during the first night check in 2013 at midwinter the occupancy rate of roosting boxes was lower than in 2012. However, we found no year difference in the effect of family size manipulation on the probability that parents claimed a roosting box, and also not in their local survival probability from midwinter to the following breeding season. This indicates that the difference in winter conditions between study years did not affect the outcome of our experiment.

Our results indicate that we were successful in inducing competition among the group of great tits that did roost in boxes because: 1) the newly available roosting boxes were immediately found and occupied (data of birds provided with a transponder in 2013), 2) the fraction of boxes occupied by great tits increased in response to the reduction in the number of roosting boxes in both years, and 3) males (the dominant sex) were more successful than females in securing a scarce roosting box in both study years. Unexpectedly in both years, with increased competition some supposedly subdominant blue tits (see: Kempenaers and Dhondt 1991) were still able to claim a roosting box and a small number
of the available roosting boxes remained empty (table 2.1). Overall, we conclude that, in both study years, we were successful in inducing competition among the manipulated great tit parents by reducing the amount of available roosting boxes.

**Survival cost of reproduction under competition**

Though we found no clear effect of family size on the ability of parents to claim a roosting box, we did find that parents that managed to claim a roosting box in plots in which we induced competition paid a survival cost of reproduction, whereas this effect was absent in the control plots.

**Dispersal or mortality?**

Important to address first is whether the observed negative effect of family size manipulation on the local survival of parents in the plots in which competition was induced was due to increased mortality or to dispersal. Great tits parents are known to have a very limited breeding dispersal (own study population: Tinbergen and Sanz 2004; Andreu and Barba 2006), but it could be that in response to the sudden drop in the number of available roosting sites parents moved elsewhere. We could not detect any movements of parents however between study plots in which competition for roosting boxes was induced and control plots, even though in the control plots empty boxes were potentially available (table 2.1). We further found no evidence for plot treatment specific effects of family size manipulation on the dispersal distance of parents between the box in which they roosted at midwinter and the box they used for breeding the next spring. This indicates that mortality effects and not dispersal effects explain the observed difference in effect of family size manipulation on the local survival rate of parents after midwinter between the experimental- and control plots.

**Experimental evidence for a survival cost of reproduction under competition**

Our results are in line, with the results of the experimental study by Nicolaus et al. (2012) in the same great tit population. In their study, competition was manipulated by experimentally altering local sex-ratios as such that male-biased, control and female-biased study plots were created. Nicolaus et al. (2012) found that a survival cost of reproduction was only paid in the male-biased, presumably competitive environments and that these survival effects occurred in the period after midwinter. Our study now points to one potential resource for which competition in the study of Nicolaus et al. (2012) occurred: the roosting box. In winter, especially males make use of roosting boxes (e.g. Křístín et al. 2001), which is also shown in our study by a higher probability to detect males roosting at midwinter. It could thus be that in the study of Nicolaus especially in the male-biased environment at midwinter competition for roosting boxes was high, leading to a survival cost of reproduction through a similar unknown mechanism as in our study.

Similar to our current study (fig. 2.3), Nicolaus et al. (2012) found that in non-competitive environments (with a female-biased sex-ratio) family size manipulation seemed to have a positive effect on parental local survival. In the study of Nicolaus et al. (2012) this positive survival effect was hypothesized to be the consequence of 1) a relaxed overall
level of competition in the local female biased environment due to an increased tendency of female fledglings to disperse and 2) a lower parental effort during post fledging care for the enlarged broods also due to a higher tendency to disperse of the female fledglings especially from enlarged broods because condition of these females was lower. In our study such an explanation does not hold because sex-ratio was not manipulated in the local environment, this suggests that other effects may be at play.

*Alternative mechanism to explain a survival cost of reproduction under competition*

We expected that parents would pay a survival cost of reproduction in our study, due to an increased proportion of parents that raised larger experimental broods having to roost outside (e.g. higher thermoregulatory costs and/or higher predation risk; Drent 1987; Mainwaring 2011). In contrast we found that experimental family size did not affect the ability of parents to claim a roosting box, but that parents in plots in which competition for roosting boxes was induced did pay a survival cost of reproduction. Our results show that survival cost of reproduction tended to be paid by those parents that claimed a roosting box after competition was induced (fig. 2.4). Potentially, depending on their experimental family size parents survived differentially as a consequence of having to defend a roosting box during the period competition was induced. Survival costs associated with defending a box could occur in the following ways: 1) directly, through injuries caused by fights or 2) through physiological/behavioural trade-offs as a result of an increased defence needed to claim a roosting box (e.g. depletion of energy reserves, changes in endocrine status, increased predation risk; e.g. Briffa & Sneddon 2007; Dufty 1989; Marler & Moore 1988). Parents that raised larger experimental broods may have suffered more injuries due to fights or the effort needed to defend their roosting box may have gone at a greater expense of their perhaps already lower energy reserves/physiological status (see appendix 1; Drent and Daan 1980; Verhulst and Tinbergen 1997; Sanz and Tinbergen 1999; Tinbergen and Verhulst 2000; Nilsson 2002; Nicolaus et al. 2012a; de Jong et al. 2014). In turn this could have led to the observed survival cost of reproduction.

The pattern that parents that raised larger experimental broods that did claim a box had almost the same value of local survival as parents of the same manipulation group that roosted outside raises the question why these parents would compete for roosting boxes in the first place (fig. 2.4). One reason for this may be that roosting boxes were only removed for a short while. Perhaps the negative consequences of roosting outside on parental local survival would have been more severe if the experiment had lasted longer. The fitness pay-off for parents to compete for a roosting box may in such a situation have been greater.

*Conclusions and implications*

In contrast to our expectation, the occurrence of a survival cost of reproduction when competing for roosting boxes in winter could not be linked to a lower ability of parents to claim such a roosting box. The exact causal explanation why costs of reproduction are paid under increased competition, thus remains unknown. Yet, the results of our study strengthen the claim that the occurrence of a survival cost of reproduction depends on the
level of competition in the parents local environment (Nicolaus et al. 2012a). Under high levels of competition family size decisions can carry over to affect the future fitness of parents. This provides a potential causal explanation for the occurrence of density dependent effects on reproductive rates within populations (e.g. avian: Kluijver 1951; Perrins 1965; Both et al. 2000; Nicolaus et al. 2013; mammals: Morris 1989; Koskela et al. 1999; Bonenfant et al. 2009). Under high population density, competition could exert a selective pressure on the family size decisions of parents and thus the reproductive rates in a population. This could occur through parents adaptively lowering their family size in the face of increased competition (Nicolaus et al. 2013: reaction norms) or by selection favouring those parents with a lower investment into current reproduction. This gives insight into how competition through selection on individual reproductive behaviour could regulate population numbers.

ACKNOWLEDGEMENTS
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Appendix S1: effects of family size manipulation on parental feeding effort and the probability to produce a late brood

METHODS

Measuring parental feeding effort
Both in 2012 and 2013, we tested whether parents increased their feeding effort in response to the family size manipulation (hereafter called ‘FS manipulation’; see methods accompanying study). To this end, in 2013, the colour ring scheme was altered and all caught parents that raised a manipulated brood, received an RFID transponder ring (catching occurred when the nestlings were 7 days old, see accompanying study; transponder type: EM4102 bird PIT tag 2.6mm, manufactured by: IB technology, Eccel Technology Limited). These transponder rings enabled us to measure the effect of FS manipulation on the number of feeding visits made by each of the parents to the nest when the nestlings were 12 days old (age around which brood energy demand peaks: van Balen 1973; Tinbergen and Dietz 1994; Sanz and Tinbergen 1999). The procedure of measuring the number of feeding visits was as follows: at the nestling age of 10 days, we fitted a dummy antenna around the flight hole in the box to get the birds acquainted to this novel object. At day 11 we replaced the dummy antenna with a real antenna fitted to a data logger (type: LID665, version V804, manufactured by Dorset identification b.v.). We used the number of feeding visits made by each of the parents during the whole following day (day 12) for subsequent analyses (00:00h to 00:00h; calculated using the same protocol as in electronic appendix S1 of Nicolaus et al. (2012)).

Next to the number of feeding visits per day, we measured the effect of FS manipulation on two other indexes of parental feeding effort, the change in brood weight after FS manipulation and the number of fledglings produced. The change in brood weight was determined by during both study years, at day 6, after family size manipulation weighing all nestlings individually (mass ± 0.1 g). When the nestlings were 14 days old we individually weighed all nestlings again to determine the change in brood weight since day 6. In those cases where all nestlings had died before day 14, the change in brood weight could not be calculated and we excluded these broods from subsequent analyses (N = 13 broods). We additionally quantified whether FS manipulation increased the number of fledglings produced. These numbers were derived from fledge checks of all nest boxes 21 days after hatching. We analysed the data gathered on the number of fledglings produced twice, including and excluding all broods where parents failed to produce any fledglings (N = 21 broods).

Measuring the probability to start a late brood within the same breeding season
Using our standard protocol during the breeding season, late broods could be determined (see section in accompanying study: ‘monitoring egg laying and breeding’). Breeding females were either identified while sitting tight on eggs or when caught at the nest when the nestlings were 7 days old (see: accompanying study). From this we could calculate the
probability of females ($N = 164$) to produce a late brood within the same season ($N = 34$; defined here as either a repeat brood: after an unsuccessful first brood or a second brood: after a successful first brood). We tested the effect of FS manipulation on the probability of females to start a late brood including and excluding the repeat broods, to check how the repeat broods ($N = 5$) affected our results. We did not focus on males because it more often occurred that they could not be caught at late broods (own population data).

**Statistics**

We analysed the effects of FS manipulation on the change in brood weight using a linear mixed effects models (LMER) with a Gaussian error structure. The effect of family size manipulation on the number of visits made by each parent to the nest and the number of fledglings produced were done using a generalized linear mixed effects model (GLMER) with a Poisson error structure. The effect of FS manipulation on the probability of parents to start a late brood was analysed using generalized linear mixed effects models (GLMER) with a Binomial error structure.

Within all analyses, FS manipulation was included as a continuous variable because of our directional expectation (directional statistical tests, see: Knowles et al. 2009, Fokkema et al. 2016) and we allowed for non-linear effects by including a quadratic effect of FS manipulation. Next to this, we included three other predictor variables: 1) study year, this factor was included in all analyses except the analysis of the number of visits/day made by each parent, as we only had one year of measurements, 2) sex of the parent, this factor was included in the analyses of the number of visits per day made by each parent. Sex of the parent was not included in the other analyses as these were done at the brood level. We included two random variables: 1) ‘trio’ number, this factor was included as a random effect in all analyses to correct for non-independence of the matched trios of nests (see methods accompanying study: ‘family size manipulation’), 2) brood ID, this identification number for the brood raised, was included as a factor in our analyses of the number of visits/day by each parent to account for the non-independence within breeding pairs. Model selection was done using a backwards elimination procedure similar as reported in the accompanying study.

**RESULTS**

**Family size manipulation and parental feeding effort**

Two of three measured indexes of parental effort increased with FS manipulation. Between years the relationship between FS manipulation and the number of fledglings produced differed (table S1; FS manipulation$^2$× year 2013: intercept: $1.94 \pm 0.09$, $\beta$: $0.05 \pm 0.02$, $\chi^2_{d.f.1} = 9.38$, $P < 0.01$, FS manipulation × year 2013: $\chi^2_{d.f.1} = 0.53$, $P = 0.47$). But, on the whole, FS manipulation had a positive effect of the number of fledglings produced (FS manipulation: $\beta = 0.09 \pm 0.01$, $\chi^2_{d.f.1} = 40.79$, $P < 0.001$). When the analysis of the effect of FS manipulation on the number of fledglings produced was run excluding broods in which no nestlings fledged, the difference between years in the quadratic effect of
family size manipulation disappeared (FS manipulation$^2 \times$ year 2013: intercept: 2.01 ± 0.07, $\beta$: 0.02 ± 0.02, $\chi^2_{df,1} = 1.99$, $P = 0.15$). All other effects were similar.

The number of feeding visits per day of both parents in 2013 also increased with FS manipulation, but the effect differed between the sexes. For males the effect of FS manipulation on the number of feeding visits was stronger than for females and did not level off at the enlarged broods (FS manipulation $\times$ sex: males: intercept: 5.67 ± 0.11, $\beta$: 0.03 ± 0.006, $\chi^2_{df,1} = 26.25$, $P < 0.001$; FS manipulation$^2 \times$ sex: males: $\beta$: 0.05 ± 0.003, $\chi^2_{df,1} = 185.88$, $P < 0.001$).

The change in brood weight between day 6 and day 14 days was positively but not significantly affected by FS manipulation (FS manipulation: intercept: 52.23 ± 3.39, $\beta$: 1.16 ± 0.85, $\chi^2_{df,1} = 1.86$, $P = 0.17$).

**Family size manipulation and the probability of females to start a late brood**

In 2013, but not in 2012, FS manipulation negatively affected the probability of females to start a late brood within the same breeding season (repeat- and second broods; FS manipulation $\times$ year 2013: intercept: −1.75 ± 0.30, $\beta$: −0.39 ± 0.17, $\chi^2_{df,1} = 4.98$, $P < 0.05$). There was no evidence for a non-linear effect of FS manipulation on the probability to produce a late brood (FS manipulation$^2$: $\chi^2_{df,1} = 0.55$, $P = 0.35$; FS manipulation$^2 \times$ year 2013: $\chi^2_{df,1} = 1.40$, $P = 0.24$). When the repeat broods were excluded from the analysis (e.g. only parents with successful first broods included) effects were similar.
PART II:
Parental competitive ability in spring