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Family planning under social competition

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Does family size affect parental competitive ability: a nest box removal experiment

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ABSTRACT

The trade-off between current- and future reproduction is a theoretically well-established concept. However, empirical evidence for the occurrence of a fitness cost of reproduction is mixed. Evidence indicates that parents only pay a cost of reproduction when local competition is high, and hence with low competition no cost is found. In line with this, recent experimental evidence from great tits showed that reproductive effort negatively affected the competitive ability of parents, estimated through competition for high quality breeding sites in spring. In the current study we further investigate the negative effect of reproductive effort on parental competitive ability in spring with the aim to better quantify the potential consequences for parental fitness. To this end, we 1) manipulated the family size of great tit (*Parus major*) parents and 2) induced severe competition for nest boxes among the parents just before the following breeding season. In response to our family size manipulation parents did increase their feeding effort and we successfully induced competition among the parents the following spring. Against our expectation we found no effect of family size on the ability of parents to secure a scarce nest box for breeding. However, in the experimental year parents paid a survival cost of reproduction before the onset of the experiment in early spring, which likely selected against individuals with lowest competitive ability. In previous years, if detected, the survival cost of reproduction was always paid after midwinter. Winter food availability during the study year was exceptionally low and thus competition in early winter may have been extra high. We therefore hypothesize that increased reproductive effort negatively affected parental competitive ability but that this year the cost of reproduction was already paid before the onset of the experiment.

INTRODUCTION

One of the corner stones of life-history theory is the cost of reproduction: an increase in current reproduction goes at the expense of fitness prospects that will be gained from future reproduction (Williams 1966, Barnes and Partridge 2003). Parents with high reproductive investment can pay this cost either by a decreased survival probability, a reduced future fecundity or both. The higher the fitness costs of reproduction, the more the parents are selected to lower their reproductive investment (Daan and Tinbergen 1990, Roff 1992, Stearns 1992).

Empirically, the evidence for the occurrence of a fitness cost of reproduction is ambiguous. Though negative effects of reproductive investment on future reproduction like second or repeat broods of the parent in the same season are relatively well established, the evidence for specifically a survival costs of reproduction is scant (birds: 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).

One reason for the mixed evidence for survival costs of reproduction is that studies on the occurrence of fitness costs of reproduction have mostly ignored the role of the environment (as also pointed out by: Nicolaus et al. 2012a, Svensson and Sheldon 1998, Ricklefs 2000). Variation between studies in finding evidence for costs of reproduction could be due to temporal and spatial variation in ecological conditions.

Testing whether costs of reproduction are indeed modulated by the environment involves experimentally manipulating not only the reproductive investment of a parent, but also the conditions in the parents' environment. This was done by Nicolaus et al. (2012) who simultaneously experimentally manipulated the family size that great tit (*Parus major*) parents had to raise and the levels of local intraspecific competition. Subsequently they measured the existence of fitness cost of reproduction. Nicolaus et al. (2012) found that survival costs of reproduction were only paid in environments with high levels of competition. These survival effects occurred after midwinter and thus well after the breeding season. The level of competition within a parents' (future) social environment may thus be an important determinant of whether or not it pays a survival cost of reproduction. Nicolaus et al. (2012) hypothesized that family size negatively affected the competitive ability of parents and only under high levels of competition survival cost of reproduction were subsequently paid. Such carry-over effects of family size on parental competitive ability and subsequent parental fitness could explain the observed negative relationship between parental reproductive investment and population density (e.g. birds: Kluijver 1951; Perrins 1965; Both et al. 2000; Nicolaus et al. 2013; mammals: Morris 1989; Koskela et al. 1999; Bonenfant et al. 2009). At high population density and presumably competition, parents may be selected to lower their reproductive investment, not just because resources for reproduction per capita decline, but also because the penalties for future survival are severe if they compromise their competitive ability.

In two separate experimental studies we have put the hypothesis that family size negatively affects parental competitive ability to the test. Within a nest box breeding great tit population, we measured the long-term effect of manipulated family size on the ability

of great tit parents to compete in the ensuing winter for roosting boxes (Fokkema et al. 2017) and in spring for preferred deeper nest boxes (Fokkema et al. 2016). In the winter period we found no evidence for a negative effect of family size on the ability of parents to claim a roosting box, but in spring we did find that family size negatively affected the ability of great tit parents to claim a preferred deeper nest box. In a follow up study focused on blue tits (*Cyanistes caeruleus*) we found that deeper nest boxes offered higher breeding success, especially in areas with high predation pressure (see: chapter 5). Hence, our experiments supported the hypothesis that a higher reproductive effort reduced parental competitive ability in the next breeding season, which potentially reduced parental fitness.

In the current study we aim to further investigate the consequences of a negative effect of family size on parental competitive ability in spring under more severe competitive conditions than our previous experiment. We study how family size affects the probability of getting a breeding box when the availability of breeding boxes was strongly reduced. Competition in this case is more a zero-sum game, as failing to obtain such a nest box means that individuals unlikely can breed, as natural nest holes are scarce in our study area. During the breeding season we manipulated the family size that great tit parents had to raise and just before the onset of the following breeding season, we drastically reduced the number of nest boxes available for breeding in the whole study area. We expected that this reduction would result in high competition for nest boxes. Under such high competition, we expected that family size manipulation would negatively affect the ability of parents to claim a nest box for breeding.

METHODS

Study area

The study was carried out in a nest-box-breeding population of Great tits in the Lauwersmeer area, in the northern part of the Netherlands (53°23'N, 6°14'E). The study area was reclaimed from the Wadden sea in 1969 after which parts were planted with deciduous trees and some conifers. The measurements took place in 12 plots of roughly 10 ha distributed over the forests (for map see: Nicolaus et al. 2009). Before the nest box removal experiment, each plot contained 50 nest boxes attached to trees at breast height (approx. 1.20 m), separated 50 m from each other in a grid. The 600 wooden nest boxes were made of 2 cm thick plywood with inside dimensions of approximately: length, width, height: 12, 8, 24 cm (for further details see chapter 4).

Family size manipulation

During the breeding season of 2014 nest boxes were checked weekly to monitor the progress of nest building and egg laying. As soon as breeding had commenced (warm and uncovered eggs in the nest cup) we could calculate the expected hatching date assuming one egg was laid per day and breeding took at least 12 days (de Heij et al. 2006). Starting two days before the expected hatching date nests were checked daily until the first eggs

hatched. When the nestlings were five days old we visited all nests and recorded the number of nestlings and the weight of the brood. Using this information we formed 'trios' of nests with similar initial conditions (clutch size, brood size and weight of the nestlings) within which we performed our family size manipulations when the nestlings were six days old. Nest treatment and nestlings to be exchanged were randomly assigned. Within most matched trios, we exchanged three nestlings from one brood (reduced) to another brood (enlarged) and kept a third nest as control (34 trios). To disturb each nest to a similar extent we also exchanged two nestlings of the reduced brood to the control brood and vice versa and repeated this procedure for the enlarged brood. For three matched trios, we exchanged two nestlings instead of three to prevent desertion of the brood by the parents (initial family size of trios: 6–7 nestlings; Verboven et al. 2002; for further details on manipulation scheme see: Fokkema et al. 2016; de Jong et al. 2014). In total over the breeding season we manipulated the original family size of 111 broods.

Parental feeding effort

We subsequently measured the effect of family size manipulation on parental feeding effort based on four components: 1) the number of visits made per day by each parent to the nest box, 2) the change in the weight of the brood after family size manipulation, 3) the total produced brood weight and 4) the number of fledglings produced.

To measure the visits made by each parent to the nest box, we made use of an RFID transponder ring (type: EM4102 bird PIT tag 2.6mm, manufactured by: IB technology, Eccel Technology Limited). Some parents that raised a manipulated brood had already been provided with a transponder ring in 2013 (N = 26) for a different study, but most had none. Therefore we caught all parents (including the previously transpondered parents) in the nest box using spring traps the day after family size manipulation. In case we failed to catch one or both of the parents we tried again two days later. In total we caught and provided 208 of the 222 parents that raised manipulated broods with a transponder ring (10 parents were either caught but forgotten to be provided with a transponder ring or failed to be caught but identified with binoculars on the basis of existing identification rings; four parents could not be caught or identified based on existing identification rings). Transponder rings were applied on the left or right leg of the parent, on the other leg an aluminium ring with unique inscription and a coloured plastic ring were attached in a unique combination. To measure the number of visits made by each parent to the nest box we used transponder readers (type: LID665, version V804, manufactured by Dorset identification b.v.). A dummy antenna mimicking the real antenna of the transponder readers was fitted on the inside of the box around the entrance hole when the nestlings were 10 days old to get the birds acquainted to this novel object. Next at day 11, we replaced the dummy antenna with the real antenna fitted to the reader and measured the number of feeding visits made by each parents during the whole following day (day 12; age around which brood energy demand peaks: van Balen 1973; Tinbergen and Dietz 1994; Sanz and Tinbergen 1999). From this data, we calculated the number of feeding visits made by each parent during the whole day (following: Nicolaus et al. 2012a, Fokkema et al. 2016).

To measure the change in weight of the brood after family size manipulation we weighed the brood two times, one time right after family size manipulation (nestlings 6 days old) and the other time when the nestlings were 14 days old. The brood weight measured when the nestlings were 14 days old was additionally used in our analysis of the effect of family size manipulation on the total brood weight produced. The number of fledglings produced was determined by daily fledge checks of the manipulated broods 21 days after hatching. When the brood had fledged, nest material was carefully inspected for any dead nestlings. The number of fledglings was determined by subtracting the number of nestlings seen when the nestlings were 14 days old with any dead nestlings found later in the nest.

The probability of a late brood

During the breeding season we recorded which females that raised a manipulated brood started a late brood later during the same breeding season. We define a late brood here as either a repeat brood (first brood did not produce any fledglings) or a second brood (first brood did produce at least one fledgling). Females could be identified during incubation while sitting tight on eggs, based on the previously applied colour ring combination or additionally when we caught the female at the nest when the nestlings were 7 days old (all females were caught; same catching procedure as in section above). We also attempted to catch males at the late broods. For 23 of the 34 pairs that started a late brood the male was identified. Of these 23 pairs, 20 females were paired with the same male as in the early brood. The females that switched males originated from 1 reduced, 1 control and 1 enlarged first brood. Males were not observed to start a late brood with a different female. Due to the fact that males were less easy to catch at late broods and there was no difference between the family size manipulation groups in the tendency of females to switch from their original partner, we decided to focus on identified females at late broods only to determine the probability to start a late brood.

Local survival of parents

We measured the effect of family size manipulation on parental local survival over two different periods: 1) from the breeding season until midwinter and 2) from midwinter until the onset of our nest box removal experiment (see figure 6.1; midwinter is defined here as the time point at which our December roost check was conducted). To accurately estimate the local survival probability of parents over these two periods we used a combination of recoveries at winter roost checks and of observations of colour ringed individuals in the study area.

Winter roost checks

Roost checks of all nest boxes in the area were performed mid-December (period 1) and mid-March (period 2; just before the nest box removal experiment). During the roost check in December all birds were taken out of the nest box and manipulated parents were identified based on the unique inscription on their previously applied aluminium ring. During the roost check in March we used handheld transponder readers (type: LID575-

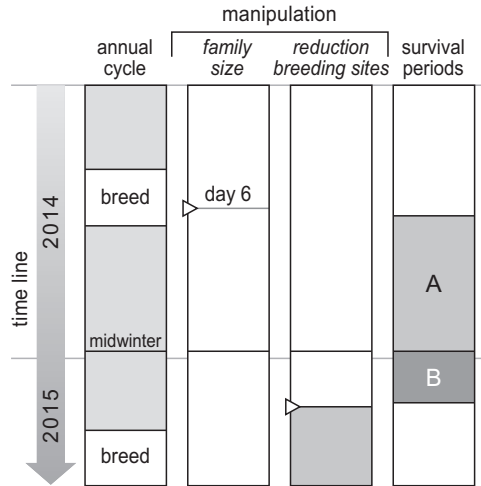


Figure 6.1: Time line of the experiment within the annual cycle. In the breeding season of 2014 when the nestlings were 6 days old family size was experimentally manipulated (second column, black line with triangle pointing right). Before the onset of the following breeding season in March 2015 we reduced the number of nest boxes available by 80 percent (third column, triangle pointing right). During the breeding season, we monitored which of the parents of the different family size manipulation groups were able to claim a breeding box. To test whether survival costs of reproduction had already been paid before competition for nest boxes was induced, we measured the local survival probability of parents over two periods: A) from the breeding season until midwinter and B) from midwinter until the onset of nest box removal experiment.

ISO; manufactured by Dorset identification b.v.) to identify roosting birds without having to open the box and handle the birds. The readers were held close to the bottom of the nest box to read the transponder identification number (previous use of these handheld transponder readers during winter roost checks in which boxes were also opened and birds taken out proved that the readers could accurately detect birds with a transponder through the bottom of the box). We used this method in March to minimize disturbance of the birds prior to the nest box removal experiment (see below).

Observations

We identified manipulated parents in the study area on the basis of observations during two weeks preceding the nest box removal experiment. We worked with a team of 4 observers. We visited each of the 12 selected study plots, 9 of the areas were visited 4–6 times and 3 of the areas 2–3 times. The latter areas were visited less because we expected less manipulated parents based on the distribution of family size manipulations in 2014 and the December roost check at midwinter. Each observation session within a plot lasted approximately 4–6 hours, during which we walked systematically through the whole area. Birds were located based on their calls and songs. To provoke the birds to call or sing we played back great tit calling sounds and songs. Once birds were spotted, we attempted to identify the birds using binoculars (8x, 10x), a telescope (20–60x or 15–45x) and/or a

camera with a long-focus lens (400 mm) on the basis of their colour ring combination (see section 'parental feeding effort'). The exact location of the birds was noted down in GPS-coordinates using a gps device (Garmin GPSmap 60 or 62s) or map.

Nest box removal experiment

During the 17th, 18th and 19th of March 2015, 80% (40 out of the 50) of the nest boxes present in each plot were removed. The 10 boxes left in the plot were randomly selected; they were cleaned and moved 35 m north-east from their original location to mitigate potential prior residency effects (Harvey et al. 1979, Andreu and Barba 2006). Whenever it was not possible to shift the boxes to the north-east, they were moved south-east, otherwise north-west or finally south-west. All the boxes were hung at breast height, facing east.

During the ensuing breeding season all remaining nest boxes were checked weekly. Breeding females were identified during incubation, while sitting tight on eggs, based on previously applied colour rings. To identify both the male and the female, additionally, we caught both parents at the nest when the nestlings were 7 days old (same protocol as specified in the section 'measuring parental feeding effort'). Next to these regular checks, in one study plot, we additionally fitted all ten nest boxes with transponder readers around the nest entrance from the beginning of the experiment onwards throughout the breeding season (type: EM4102 data logger with a EM Datalog Loop Antenna of 65mm, manufactured by: IB technology, Eccel Technology Limited). This allowed us to monitor all visits made inside the nest boxes by birds provided with a transponder, thus also those birds with a transponder that did not manage to claim a nest box.

In addition to monitoring the nest boxes we checked the area for breeding attempts in natural cavities during the whole breeding season. We conducted systematic checks of the 8 study plots in which most manipulated parents were seen preceding the experiment (see section above). We systematically surveyed the wood lots with either 1 or 2 persons. We located natural cavities in tree trunks or in branches by visual inspection of trees, hereby also making use of sounds made by birds inhabiting these cavities (alarm calls, singing or begging calls of the offspring). We played back these sounds while walking through the woodlots to provoke birds to respond (see section above). For 3 of these 8 study plots we additionally systematically surveyed (using a similar method as described above) woodlots adjacent to the study plots (within a distance of 1 kilometre from the plot) where we suspected that the trees in these areas would have natural cavities (more large poplar (*Populus spp.*) trees and more dead trees present, e.g. Newton 1994)). Besides these systematic checks, in an opportunistic way during our normal rounds of the whole area we also paid extra attention in the remaining 4 study plots and the woody areas between our study plots for natural cavities.

If natural cavities were located we used a ladder and an endoscope (type: Basetech BSK-100) to determine if the cavity was occupied and, if possible, by which species or, in the case of great tits, by which individual bird (based on existing identification rings, see above). If unattended great tit broods (based on the appearance of the eggs) were detected we attempted to identify the breeding birds from a distance with binoculars based on their existing identification rings.

Analysis

All data analysis was done in the program R (version 3.3.1). Figures were created using the package ggplot2 (Wickham 2009) and the predicted lines in the figures 6.2–4 were created using the predict function. Effects of family size manipulation on the number of visits made by each parent to the nest box and the number of fledglings produced, were analysed using a generalized linear mixed model with a Poisson error structure. The effect of family size manipulation on the weight change of the brood after manipulation and the brood weight at day 14 were analysed using a linear mixed model with a Gaussian error structure. Generalized linear mixed models with a binomial error structure were used to analyse variation in the probability to start a late brood, to survive until midwinter, to survive from midwinter until March and the probability of parents to claim a breeding box.

The goal of our analyses of the effect of family size manipulation on parental local survival was to test whether parents of the different manipulation groups differed in local survival before the onset of our nest box removal experiment. We decided that mixed models were most suitable to do our analyses because an analysis of the same dataset with mark recapture models using the program MARK (White and Burnham 1999) showed that there was no significant difference in the recapture probability of the parents of the different manipulation groups (average recapture probability \pm S.E: period 1 from the breeding season until the roost check in December: 0.75 ± 0.06 ; period 2 from December until the roost check in March: 0.81 ± 0.00 ; $c\text{-hat} < 0$, thus we assumed that $c\text{-hat} = 1$ following the advice in the MARK manual). Further by using mixed models we could correct for non-independency in our dataset, whereas in the program MARK we could not do this (see: ‘random effects included’). Here we thus report on the effect of family size manipulation on the apparent survival of parents assuming the recapture rates of all parents to be 1. The outcome of our mark recapture models was consistent with our mixed models (the most supported model included family size manipulation as predictor for local survival).

Response variables included

Parental feeding effort

For the analysis of the effect of experimental family size on parental feeding effort the change in brood weight could only be calculated for those broods where nestlings were still alive at day 14 ($N = 98$ out of 111 broods). We therefore also did our analysis of the number of fledglings produced including ($N = 111$ broods) and excluding all nests that failed to produce any fledglings ($N = 93$, 18 broods failed of which 5 failed after day 14). The analysis of the effect of experimental family size on the number of visits made by each parent was done for 54 parents at 27 broods at which we measured feeding frequency (10 Reduced broods, 8 Control broods, 9 Enlarged broods). Nine broods could not be included because measurements were incomplete due to failure of the readers or because the brood had died at day 12 when the number of visits was measured.

The probability of parents to start a late brood

We did our analysis of the probability of all identified females in the first broods (N = 110) to start a late brood two times, including as late brood either both repeat and second broods (N = 34 late broods) or only second broods (N = 31 late broods).

Parental local survival

Parental local survival was calculated over two periods (see fig. 6.1). For the first period from the breeding season (N = 218 identified manipulated parents) until midwinter, we considered as midwinter survivors all manipulated parents seen during the roost check in December (N = 74 survivors). For the second period from December to March we considered as spring survivors all parents (of the group of parents observed roosting in December) seen during the March observations or the March roost check (N = 43 survivors; N = 9 parents were exclusively seen in the March observations and N = 9 parents were exclusively seen in the March roost check).

The probability of parents to claim a breeding box

27 of the in total 57 identified parents in the March observations and roost check occupied a breeding box the following breeding season. We analysed the effect of family size manipulation on the probability to claim a breeding box in 2015 on the basis of this sample. Four of the parents claimed to be successful, were only seen at breeding attempts in nest boxes late in the breeding season (44, 56, 59 and 59 days after the first egg was laid in the population). To check whether inclusion of these four parents affected our conclusion, we redid the analysis including these parents coded as not successful. This did not change our conclusion.

Predictor variables included

We included family size manipulation as a continuous variable in all analyses as we aimed to test the direction of the effect of family size manipulation (directional statistics, as in Fokkema et al. 2016). This additionally allowed us to include a quadratic effect of family size manipulation, to model any non-linear effects (termed family size manipulation² hereafter).

We included sex of the parent to explain variation in the number of visits made by each parent per day, in the local survival of parents until- and after midwinter and in the probability of parents to claim a breeding box. We included sex of the parent as main effect and in interaction with family size manipulation and manipulation².

Random effects included

To correct for non-independence between the nests within the manipulation trios we included the trio of nests between which nestlings were exchanged during family size manipulation as a random effect in all analyses.

Model selection

We used a backwards elimination procedure for model selection based on likelihood ratio tests. If included in the model, we first tested whether the interaction between manipula-

tion² and sex of the parent could be eliminated. Next we tested if the interaction between family size manipulation and sex of the parent could be eliminated. Then we proceeded by testing whether family size manipulation² could be eliminated and finally we tested whether sex of the parent and family size manipulation could be eliminated. The quadratic term of family size manipulation (family size manipulation²) was never left in the model without the linear term of family size manipulation. We kept the random effect of trio-number in the model at all times to correct for non-independence in the dataset.

RESULTS

Parental feeding effort

Family size manipulation increased parental feeding effort based on three out of the four measured indexes. The number of visits per day increased in a non-linear way with experimental family size for both males and females, but in females we found an accelerating slope, whereas in males the slope was decelerating with experimental family size (table 6.1; fig. 6.2). We further found that the number of fledglings produced increased significantly with family size manipulation (intercept: 1.73 ± 0.06 , family size manipulation: $\beta = 0.05 \pm 0.02$, $\chi^2_{d.f.1} = 8.86$, $P < 0.01$). This effect did not level off for the enlarged broods and further did not differ depending on whether broods in which no nestlings were produced were included in the sample. In contrast, we found no effect of family size manipulation on the growth in brood weight after manipulation (intercept: 48.04 ± 3.90 , family size manipulation: $\beta = -0.31 \pm 1.06$, $\chi^2_{d.f.1} = 0.08$, $P = 0.77$). The total weight of the brood measured in the week before fledging (day 14) did increase significantly with family size manipulation (intercept: 7.80 ± 1.07 , family size manipulation: $\beta = 7.80 \pm 1.07$, $\chi^2_{d.f.1} = 39.01$, $P < 0.001$). The latter indicates that for the enlarged broods per nestling

Table 6.1: Outcome of the mixed model estimating the effect of family size manipulation on the number of feeding visits made by each parent (male and female) to the nest. The number of visits per day increased non-linearly with experimental family size for both sexes. The effect of experimental family size did differ between the sexes (see also fig. 6.2).

| Variable | Estimate β (\pm SE) | Chi2 | d.f. | P |
|---|------------------------------|-------|------|--------|
| intercept | 4.96 (0.16) | | | |
| family size manipulation | 0.07 (0.005) | | | |
| sex | | | | |
| male (relative to female) | 0.46 (0.03) | | | |
| family size manipulation ² | 0.04 (0.004) | | | |
| family size manipulation x sex | | 36.51 | 1 | <0.001 |
| family size manipulation x sex: male | 0.05 (0.007) | | | |
| family size manipulation ² x sex | | 208.8 | 1 | <0.001 |
| family size manipulation ² x sex: male | -0.06 (0.004) | | | |

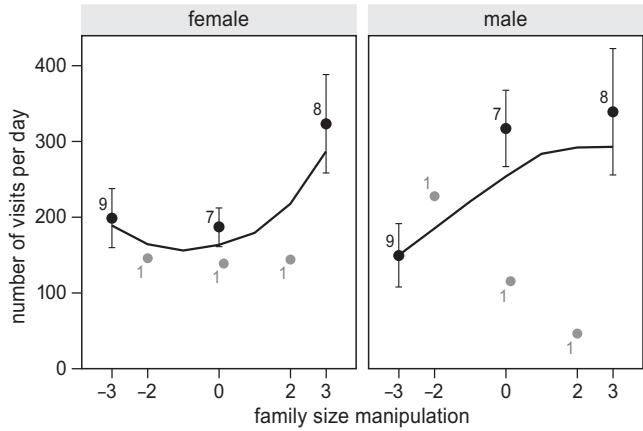


Figure 6.2: The effect of family size manipulation on the number of feeding visits per day made by the female (left part of graph) and the male (right part of graph) parents. For both male and female the number of feeding visits increased with family size manipulation, but the shape of the response differed between the sexes (for analysis see table 6.1).

the growth was less relative to the control and reduced broods, but in total parents of the enlarged broods were able to maintain the higher brood weights created after manipulation.

The probability to produce a late brood

We found a clear negative effect of family size manipulation on the probability of females to start a late brood during the same breeding season (fig. 6.3A; intercept: -0.99 ± 0.27 , family size manipulation: $\beta = -0.29 \pm 0.10$, $\chi^2_{d.f.1} = 9.39$, $P < 0.01$). We found no evidence that the effect was non-linear.

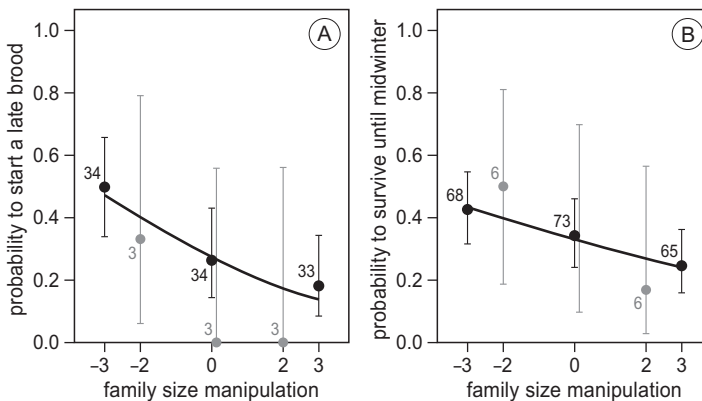


Figure 6.3: The effect of family size manipulation on A) the probability of females to start a late brood within the same breeding season and B) the local survival probability of both male and females from breeding till midwinter. Family size manipulation had a negative effect on both fitness components.

The probability of parents to survive until and after midwinter

We found that family size manipulation negatively affected the local survival probability of parents until midwinter (period 1: fig. 6.1; results see: fig. 6.3B; intercept: -0.70 ± 0.16 , family size manipulation: $\beta = -0.15 \pm 0.06$, $\chi^2_{d.f.1} = 5.85$, $P < 0.05$). We found no evidence that the effect of family size manipulation was non-linear nor that it differed between the sexes.

We found evidence for a non-linear negative effect of family size manipulation on the local survival probability of parents from midwinter until March (period 2: fig. 6.1; results see: table 6.2, fig. 6.4). The survival effect after midwinter caused by family size manipulation was less clear and we found that it differed between the sexes, with a more pronounced negative effect in females than males (table 6.2, fig. 6.4).

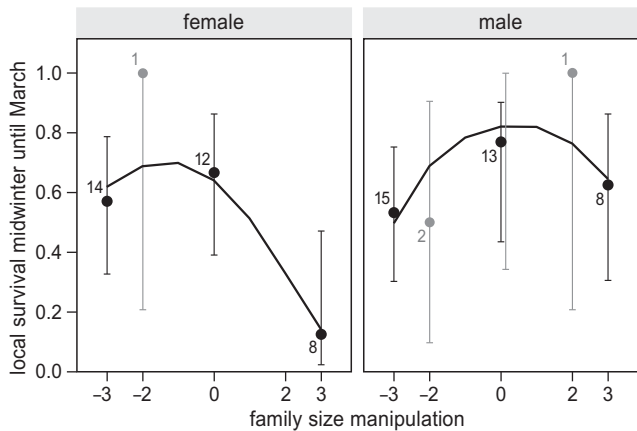


Figure 6.4: The effect of family size manipulation on the local survival probability of both parents from midwinter until the onset of our nest box removal experiment. Analysis showed a strong trend that females paid a survival cost of reproduction, whereas males did not.

Table 6.2: Outcome of the mixed model estimating the effect of family size manipulation on the probability of each parent to survive from midwinter until March. The inear component of the slope differed between the sexes. The quadratic effect of family size manipulation did not differ (see also fig 6.4).

| Variable | Estimate β (\pm SE) | Chi2 | d.f. | P |
|---|------------------------------|------|------|--------|
| intercept | 0.60 (0.57) | | | |
| family size manipulation | -0.39 (0.20) | | | |
| sex | | | | |
| male (relative to female) | 0.97 (0.61) | | | |
| family size manipulation ² | -0.14 (0.07) | 4.47 | 1 | < 0.05 |
| family size manipulation x sex | | 4.28 | 1 | < 0.05 |
| family size manipulation x sex: male | 0.49 (0.27) | | | |
| Rejected terms: family size manipulation ² x sex (d.f.1) | | | | |

Table 6.3: Outcome of a mixed model estimating the effect of family size manipulation on the ability of parents to claim a nest box after we induced competition. We found a negative but non-significant effect of experimental family size on the ability of parents to claim a nest box the following breeding season.

| Variable | Estimate β (\pm SE) | Chi2 | d.f. | P |
|--|------------------------------|------|------|------|
| intercept | -0.42 (0.28) | | | |
| family size manipulation | -0.06 (0.13) | 0.18 | 1 | 0.67 |
| Rejected terms: | | | | |
| family size manipulation ² x sex (d.f.1), family size manipulation x sex (d.f.1), family size manipulation ² , sex (d.f.1) | | | | |

Nest box removal experiment

Signs of increased competition

We found several indications that competition in 2015 after removal of 480 of the 600 nest boxes indeed increased. When we compared 2015 with 2014, a recent high population density year, we found that the fraction of nest boxes occupied by great tits significantly increased (from 0.42 in 2014 to 0.91 in 2015; chi-square goodness of fit test: $\chi^2_{d.f.1} = 118.8$, $P < 0.05$). The fraction of nest boxes occupied by subdominant blue tits (e.g. see: Lohrl 1977, Kempenaers and Dhondt 1991, Dhondt and Adriaensen 1999) drastically decreased (from 0.29 in 2014 to 0.08 in 2015; chi-square goodness of fit test: $\chi^2_{d.f.1} = 22.68$, $P < 0.05$).

Registrations using transponder readers in all nest boxes of one plot throughout the 2015 breeding season further show that there were more birds visiting the nest boxes than actually bred in one (table 6.4). In total we recorded 12 unique individuals with a transponder in the monitored study plot, five of these individuals claimed and bred in one of the available nest boxes and were not registered in nest boxes other than in which they bred. The other seven recorded individuals with a transponder were not observed as breeder in any of the nest boxes, but did visit several (up to 6) of the available nest boxes in the plot. Most visits by individuals that did not claim a box occurred before the first egg was laid in the focal nest box, but in several cases they also occurred later. While it must be noted that we have no comparison of transponder data gathered in previous years, the above transponder data does show that evidently more individuals were interested in the available nest boxes (11 of the 12 transpondered individuals seen, had bred in the previous year in the monitored study plot; individual number 3 had bred in the previous year, but in another study plot approximately 2 kilometres away).

Additionally, we found 50 suitable cavities as alternative to nest boxes in the study area and that some great tit parents that used nest boxes for breeding in 2014 switched to these cavities. In the whole area we detected 50 occupied natural cavities, 18 occupied by great spotted woodpeckers (*Dendrocopos major*), 17 by great tits, 7 by blue tits, 1 by a spotted flycatcher (*Muscicapa striata*), 1 by the common redstart (*Phoenicurus phoenicurus* while of 6 cavities the species of the breeding pair could not be identified (the surface area of the study area was approximately 24 km²). 16 great tits breeding in natural cavities could be identified based on existing identification rings, 6 of them bred in

our nest boxes in 2014, 5 individuals were first year breeders fledged from one of the nest boxes in 2014, 3 fledged from our boxes before 2014 and 2 had been detected in our nest boxes during winter roost checks in 2013 but were never detected in the breeding season. The fact that 6 parents which previously bred in boxes in 2014 now bred in cavities indicates that competitive displacement of parents indeed occurred as a consequence of our nest box removal experiment.

The probability of parents to claim a nest box

We found a negative but non-significant effect of previous family size manipulation on the probability of parents to claim a breeding box after competition was induced (table 3; average probability of manipulated parents to claim a box: Reduced: 0.59 (95% CI: 0.39, 0.78), Control: 0.36 (95% CI: 0.20, 0.55), Enlarged: 0.50 (95% CI: 0.24, 0.76); sample sizes respectively: 22, 25, 10; -3/-2 and +2/+3 manipulations grouped for comparison).

Table 6.4: Overview of the number of visits made by individual great tits with transponder rings to nest boxes in one of our study plots throughout the breeding season. The first two columns depict the individual and its sex respectively. The next 10 columns depict each nest box in the study plot and the values in the cells represent the number of visits each individual made to that particular box. All nest boxes depicted here were occupied by breeding pairs, but not all individuals had a transponder. If the cells are high lighted this means that this was the box in which the individual was observed breeding. Note that individuals that did not breed in any of the boxes in the study area visited more different boxes. For those individuals we have depicted when the visits took place in the last three columns: before the onset of laying, after the onset of laying and the date of the last registration of the bird in one of the boxes in the plot (the average laying date of first broods in the monitored plot was 28/04/15). *Individual 1 bred in two nest boxes, her first brood in box 6 failed and she laid a repeat clutch in box 8. She and her partner (without a transponder) were however then displaced while breeding by the former owners that started a second brood in box 8.

| Individual | sex | Nest box | | | | | | | | | | Timing of visits | | date last registration | | |
|------------|-----|----------|---|---|------|-----|-----|------|-----|-----|----|--------------------------------|-------------------------------|------------------------|----------|--|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | visit before clutch initiation | visit after clutch initiation | | | |
| 1* | F | | | | | | 390 | | 122 | | | | | | | |
| 2 | M | | | | | 986 | | | | | | | | | | |
| 3 | F | | | 1 | 4 | 1 | | | 1 | 2 | 2 | 11 | 0 | 15/03/15 | | |
| 4 | M | | | | | | | 168 | | | | | | | | |
| 5 | M | | | | | 1 | 15 | | | 6 | 12 | 16 | 18 | 10/05/15 | | |
| 6 | F | 1 | | | 63 | | 12 | 79 | | | | 148 | 7 | 06/05/15 | | |
| 7 | F | | | | | | | 1759 | | | | | | | | |
| 8 | M | | 6 | | | 1 | 1 | | | | | 6 | 2 | 03/05/15 | | |
| 9 | M | | | | 1862 | | | | | | | | | | | |
| 10 | F | | 1 | | | 4 | | | | 170 | 26 | 195 | 6 | 22/05/15 | | |
| 11 | F | | 7 | 2 | 1 | | | | 2 | 3 | 13 | | 8 | 20 | 09/05/15 | |
| 12 | F | | 6 | 1 | 1 | | | | | | | | 7 | 1 | 30/04/15 | |

DISCUSSION

Our aim was to test if family size negatively affected the future ability of great tit parents to compete for scarce nest boxes in a large-scale nest box removal experiment. Such effects of family size on the competitive ability of parents could provide a causal explanation for why survival costs of reproduction are modulated by the local level of competition (Nicolaus et al. 2012a). In contrast to our previous work, we found no evidence that family size negatively affected the ability of great tit parents to compete for nest boxes in spring (Fokkema et al. 2016). Below we discuss potential reasons for the absence of this expected effect and stress the point that competition may affect why in some cases costs of reproduction are found, and in others not.

Methodology and sample size

Did family size manipulation increase parental feeding effort?

One general assumption of family size manipulation studies is that the energetic workload of parents increases with experimental family size (tested by e.g. Verhulst and Tinbergen 1997, Sanz and Tinbergen 1999, Tinbergen and Verhulst 2000, Nilsson 2002). A higher workload may lead to physiological costs (Zera and Harshman 2001, Ricklefs and Wikelski 2002, Speakman 2008, Hegemann et al. 2013) which may subsequently reduce parental competitive ability, potentially leading to fitness cost of reproduction (but note that other mechanisms may also be at play, see: Fokkema et al. 2016). In our study we found evidence that parental feeding effort indeed increased with experimental family size. Parents raising larger experimental broods increased their number of feeding visits and were able to sustain the higher number of nestlings and brood weight until fledging. The fact that the increase in the number of feeding visits at least for the males levelled off at the enlarged broods and that the growth in brood weight after manipulation was not affected by experimental family size indicates that parents did not completely keep up with the increased demand of their brood (Tinbergen and Verhulst 2000).

Within the same breeding season, family size manipulation had a negative effect on the probability of parents to start a late brood. Despite this latter effect we found that experimental increases in family size reduced parental local survival before and after midwinter, indicating that effects of experimental family size persisted after the breeding season. These results are in line with our previous work (Fokkema et al. 2016; Nicolaus et al. 2012a) in which we also found that despite the negative effect of experimental family size on the probability of parents to start a late brood, long-term effects of experimental family size on their competitive ability and survival in competitive environments still occurred. The existence of the negative survival effect of brood manipulation suggests that by foregoing a late brood within the same breeding season parents cannot fully escape negative effects of family size of the first brood on their future performance.

Was competition for breeding boxes successfully induced?

The following data were consistent with the interpretation that in our experimental reduction of nest boxes competition for nest boxes occurred: 1) the fraction of nest boxes

occupied by great tits drastically increased at the expense of the fraction of subordinate blue tits that markedly decreased (similar to findings of Lohrl 1977, Dhondt and Adriaensen 1999), 2) more than one breeding pair was interested in the same nest box at the same time in the breeding season and 3) at least six great tit parents which had in 2014 still bred in nest boxes were displaced to natural cavities in 2015.

With regard to the timing of the experiment one could argue that the date to reduce the number of nesting boxes was too late (mid-March, just before the breeding season). It could be that breeding pairs at this time had already decided for one breeding location and were less keen/able to move to a different territory or breeding location when nest boxes were removed (e.g. Sandell and Smith 1991, Andreu and Barba 2006). If we would have conducted our experiment in autumn when great tits also compete for territories (Drent 1983), we might have been more successful. However, an experiment that early in the year would make it hard to separate effects of experimentally manipulated family size on winter survival from effects on the ability of parents to claim a nest box for breeding, the goal of our work. Furthermore, in our previous studies we found no effects of experimental family size on the ability of parents to compete in winter for nest boxes, while we did find effects in spring, suggesting that differences in competitive ability among parents may be most important in this period (Fokkema et al. 2016, 2017).

Were fitness costs of reproduction already paid before the experiment?

We suggest that in this particular year, the survival cost of reproduction was already mostly paid before we induced competition for nest boxes, thus erasing any competitive differences resulting from the family size manipulations. The observation that survival costs of reproduction were paid in our experimental year before midwinter (fig. 6.3B) is in contrast to previous family size manipulation studies in our study area (Nicolaus 2012, Fokkema et al. 2016, 2017). A likely reason related to competition is that the availability of an important winter food of great tits in our study area, Sea Buckthorn (*Hippophae rhamnoides*) berries was exceptionally low in the winter of 2014. Local annual survival of great tits in our study area correlates positively with the winter density of sea buckthorn berries (Tinbergen et al. in prep). Similarly, Tinbergen et al. (1985) suggested that only in years with a low winter food (beech crop in that particular population) great tit parents paid a survival cost of reproduction. A similar relationship may exist between the occurrence of the survival cost of reproduction and the availability of sea buckthorn berries in our population. Perhaps, due to the very low availability of sea buckthorn berries in our experimental year, selective disappearance occurred of individuals with low competitive ability during the winter, and as a consequence we found no effect of previous reproductive effort anymore on the competition for the scarce nest boxes the following spring.

Whereas we found a clear survival cost of reproduction before midwinter, we also found that family size negatively affected the local survival of females (but not males) from midwinter until March. This difference was caused especially by females which raised an enlarged brood surviving less well after midwinter (fig. 6.4). Such a difference between the sexes was not apparent in the local survival of the manipulated parents until midwinter. Perhaps this difference between the sexes in local survival was caused by the

differences in the feeding effort of males and females in response to family size manipulation (fig. 6.2). For males, but not females that raised enlarged broods the number of feeding visits levelled off, therefore males that raised enlarged broods may have suffered lower mortality costs as a consequence of the increased family size. In previous work we never found differences between the sexes in survival in this period (Nicolaus et al. 2012a, Fokkema et al. 2016, 2017), the fact that these costs differed in our study is intriguing as it indicates a potential conflict between the sexes in terms of optimal reproductive decisions (e.g. Horak 2003, Siefferman and Hill 2008). The fact that a survival cost of reproduction was still paid, indicates that cost of reproduction had not been fully paid before midwinter.

As a consequence of the survival costs of reproduction being paid before the onset of our nest box removal experiment the sample size of especially the group of parents that raised an enlarged group became small (see results) also limiting the probability to detect effects of family size manipulation on the probability of parents to claim a nest box the following spring.

Conclusions

Our experiments set out to test the costs of reproduction through long-term negative effects of raising different family sizes on parental competitive ability. Based on previous results we expected these effects to occur mostly after mid-winter, but we failed to show that individuals raising enlarged families lost competition for scarce breeding boxes. However, our results were consistent with the hypothesis that social or ecological causes are important mediators of costs of reproduction. The naturally occurring low availability of winter food in this study potentially resulted in the low survival of individuals raising enlarged families which under mild conditions, without strong competition, may not have occurred. We want to stress that costs of reproduction may vary depending on these ecological circumstances encountered later in the year. There is a great need for studies manipulating both reproductive effort and subsequent ecological conditions, to establish these effects.

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