

Local sex ratio affects the cost of reproduction

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Summary

1. Costs and benefits of reproduction are central to life-history theory, and the outcome of reproductive trade-offs may depend greatly on the ecological conditions in which they are estimated. In this study, we propose that costs and benefits of reproduction are modulated by social effects, and consequently that selection on reproductive rates depends on the social environment.
2. We tested this hypothesis in a great tit *Parus major* population. Over 3 years, we altered parental reproductive effort via brood size manipulations (small, intermediate, large) and manipulated the local social environment via changes in the local fledgling density (decreased, increased) and the local sex ratio (female-biased, control, male-biased).
3. We found that male-biased treatment consistently increased the subsequent local breeding densities over the 3-year study period. We also found that parents rearing small broods in these male-biased plots had increased survival rates compared with the other experimental groups.
4. We conclude that reproductive costs are the product of an interaction between parental phenotypic quality after reproduction and the social environment: raising a small brood had long-lasting effects on some phenotypic traits of the parents and that this increased their survival chances in male-biased environment where habitat quality may have deteriorated (via increased disease/predation risk or intraspecific competition).
5. Our results provide the first experimental evidence that local sex ratio can affect reproductive costs and thus optimal clutch size.

Key-words: density, disease risk, intraspecific competition, optimal clutch size, *Parus major*, predation risk, reproductive trade-offs, sex ratio, social environment

Introduction

Life-history theory predicts that individuals behave in an optimal manner, and hence, parents should produce the number of offspring that maximizes their fitness (Lessells 1991; Roff 1992). Optimal reproductive investment involves trading-off both the number and quality of offspring produced (Lack 1947, 1966) as well as investment into current and future reproduction (Williams 1966; Charnov & Krebs 1974). Theory predicts that increased parental investment in current reproduction should reduce future parental fecundity and/or survival, that is, parental residual reproductive value (Williams 1966; Charnov & Krebs 1974).

The existence of reproductive trade-offs has been extensively tested in avian studies using manipulations of clutch size or brood size. In short-lived birds, these manipulations revealed that an increase of parental effort during current reproduction can affect the benefits of reproduction via

effects on the number of recruits (e.g. Tinbergen & Sanz 2004). Alternatively, or in addition, the costs of reproduction may be affected via effects on parental residual reproductive value (e.g. via reduced future fecundity in the same year: Smith, Källander & Nilsson 1987; Parejo & Danchin 2006; in the subsequent year: Røskoft 1985; Gustafsson & Sutherland 1988; or via reduced survival: de Heij, van den Hout & Tinbergen 2006; Siefferman & Hill 2008).

Despite these examples, many studies fail to show consistent support for costs or benefits of reproduction (see review in Parejo & Danchin 2006). The cause of this variation among studies is hard to pinpoint, because it could be driven by any spatio- or temporal differences in the ecological conditions in which costs and benefits of reproduction are estimated. For example, some studies have shown that parental investment into current reproduction can be manipulated by altering ecological factors of the environment such as local breeding density (Both 1998), predation pressure (Marström, Kenward & Engren 1988) or food availability (Soler & Soler 1996). It has also been suggested that

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phenotypic adjustment of reproductive decisions to ecological conditions can be adaptive and can underlie ubiquitous patterns of population regulation such as the negative density dependence of clutch size (Both, Tinbergen & Visser 2000). In this study, we are interested in social effects on reproductive costs and benefits. Social factors, particularly local densities, are likely to affect selection on reproductive rates, yet, to our knowledge, no experimental studies have tested whether this is the case. Quantifying the fitness consequences of brood size manipulation in a manipulated social environment could demonstrate that optimal reproductive investment of one individual depends on decisions made by other members of the population (Both, Visser & Verboven 1999; Mesterton-Gibbons & Hardy 2004).

In this study, we experimentally tested whether costs and benefits of reproduction are modulated by social effects in a great tit *Parus major* meta-population. We specifically focused on the effects of local fledgling density and fledgling sex ratio on the outcome of reproductive trade-offs because these social factors are expected to affect local habitat 'quality' and consequently to affect selection on optimal family size. In the great tit, males are dominant over females for food and roosting sites (Drent 1983; Wilson 1992) and are more philopatric (Greenwood, Harvey & Perrins 1979). Therefore, we assumed that intraspecific competition level or mortality risks (via density-dependent disease/predation risk) should increase with the density and/or with the proportion of dominant males in an area (Newton 1998). We predicted that optimal family size should decrease with increasing number of fledglings or with increasing proportion of males in the environment because increased competition/mortality risks might i) reduce the number of recruits per brood and thus lower the benefits of reproduction or ii) reduce future parental fecundity and/or survival and thus increase the costs of reproduction.

To test this hypothesis, over 3 years, we manipulated parental brood size, brood sex ratio and the local fledgling composition of 12 plots in a great tit meta-population. By altering the frequency of manipulated broods per plot, we affected the number of male and female nestlings raised and fledged per plot, that is, the local sex-specific densities. We then quantified the fitness consequences of reproduction (on the basis of the brood size manipulations) in relation to the manipulated local plot density (low, high) and plot sex ratio (female-biased, balanced, male-biased). We predicted optimal brood size to be smaller in high-density/male-biased plots than in low-density/female-biased plots. The interaction between workload and individual phenotypic quality may differ between the sexes, depending on whether local density and sex ratio affect both sexes equally. In great tits, males are bigger, require more energy (Hogstad 1989) and dominate females (Wilson 1992), and hence, fitness effects of increased sex-specific density may be different for males and females. If the plot manipulation affects inter-sexual competition, we expect adult females to pay higher costs of reproduction in male-biased plots, whereas males may be affected less. Alternatively, if intra-sexual competition is

more important, then we expect the majority sex to experience higher competition and to pay higher costs of reproduction.

Materials and methods

STUDY AREA AND STUDY SPECIES

The study was carried out in a great tit population in the Lauwersmeer area, located in the north-east of the Netherlands (53°23'N, 6°14'E, see map in Nicolaus *et al.* 2009b). Before the 2005 breeding season, we reorganized the existing study area by establishing 12 nest-box areas (plots). Each plot consisted of 50 boxes, spaced at 50-m intervals. The plots consisted primarily of young deciduous trees (*c.* 30 years old), and few natural cavities were available. The vast majority of the breeding attempts occurred thus in the nest boxes (mean proportion of occupied nest boxes per plot \pm SD between 2005 and 2007 = $41 \pm 12\%$, $n = 12 \times 3 = 36$ plots).

DATA COLLECTION

From the beginning of April in each year of the study, nest boxes were checked weekly, and laying date (back-calculated assuming that one egg was laid per day) and clutch size were recorded. Before the expected hatching date, nest boxes were checked daily to determine hatching date (day 0). At day 2, nestlings were bled and nail clipped for individual identification. Sexing was performed between day 3–5 using molecular markers (Griffiths *et al.* 1998). At day 6, nestlings were weighed (mass \pm 0.1 g), ringed with an aluminium ring and swapped between nests of the same age and between plots according to the experimental set-up (see below). At day 7, both parents were caught with a spring trap in the nest box, weighed, measured, ringed for later identification and externally equipped with a passive integrated transponder tag (PIT tag), if necessary (see details in Appendix S1). At day 14, body measurements of the juveniles were taken (body mass, tarsus length and length of the third primary). From day 19 onwards, boxes were checked every second day to determine the fledge date. Nests were emptied and checked for dead chicks. In 2006, additional provisioning data of parents were collected on 94 broods (see methods in Appendix S1). Further weekly checks gave information about the incidence of second clutches and their breeding success. We did not perform any experiment on the second broods (see definition in data selection), but the standard measurements of nestlings and adults were taken. Local adult survival probability, the number of recruits per nest, the laying date and clutch size the next year were estimated on the basis of recaptures and monitoring of breeding birds in the study area the next year. An overview of the breeding variables of the first broods of the three study years (2005–2006–2007) is presented in Appendix S2. Roosting inspections in winter (mid-December) gave information on which birds were still present in the study area. Identity and standard body measurements were taken for all roosting birds, and unringed birds were ringed.

EXPERIMENTAL SET-UP

To test whether local density and sex ratio modulate costs or benefits of reproduction, we altered both parental social environment and parental reproductive effort. Plot nestling density (number of nestling per plot) and plot sex ratio (proportion of male nestlings per plot) were altered with the aim to manipulate local juvenile density and sex ratio over an extended period after fledging. We manipulated

parental reproductive effort via brood size manipulations. We performed these manipulations in three consecutive years (2005, 2006 and 2007) (Appendix S2, see below).

Brood manipulations were carried out at day 6 such that broods were categorized into 'small', 'intermediate' or 'large' broods and as 'female-biased', 'control' or 'male-biased' broods (Fig. 1). We defined 'intermediate' broods as being equal to the average population brood size of a given year and 'small' and 'large' broods as broods that differed respectively by -3 or $+3$ nestlings from the average population brood size of a given year (e.g. in 2005, 'small' = 5 nestlings, 'intermediate' = 8 nestlings and 'large' = 11 nestlings). We defined 'female'-biased, 'control' and 'male'-biased broods as broods we manipulated to contain *c.* 25%, 50% and 75% of nestling males, respectively (Fig. 1). The brood manipulations (brood size and brood sex ratio) were performed with the goal of manipulating parental choices. Brood sex ratio and brood size manipulation were

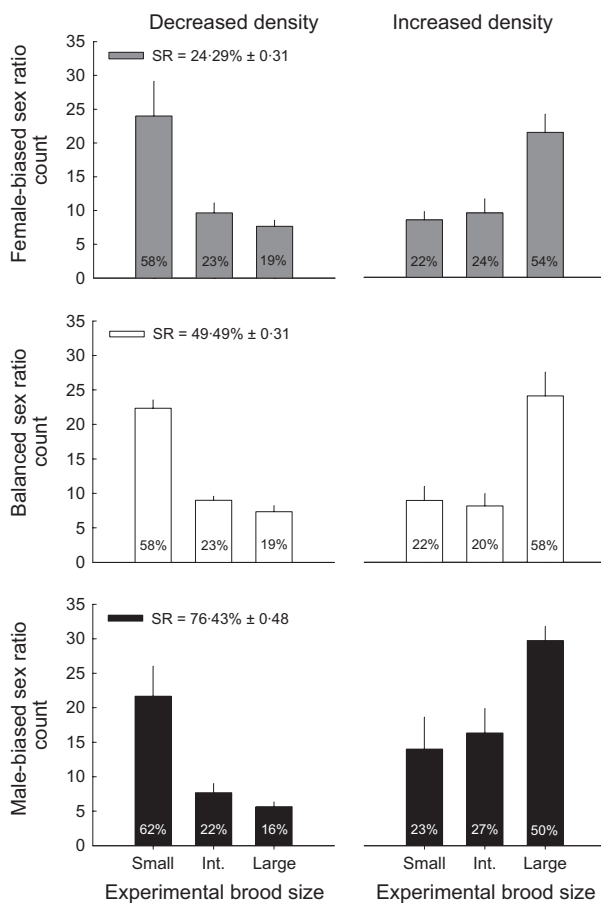


Fig. 1. During three consecutive years (2005–2007), nestling plot density and sex ratio were manipulated in the Lauwersmeer great tit population by altering the frequencies of the different brood treatments in a specific direction within each of the 12 plots. To achieve the plot density treatments (decreased/increased), *c.* 60% of the brood size was manipulated in the direction of the plot treatment while keeping *c.* 20% of the broods manipulated towards the opposite brood size treatment and *c.* 20% of the broods towards intermediate brood size (% indicated in bars). To achieve the plot sex ratio treatments, all broods within a plot were manipulated in the direction of the plot treatments [*c.* 25%, 50% or 75% of nestling males in female-biased (grey), balanced (white) and male-biased (black) plot sex ratio treatments, respectively]. Mean nest counts and mean sex ratios are presented with their standard error.

thus performed outside 'individual ranges' but within the 'natural ranges' of the species (Nicolaus *et al.* 2009b). We assigned all the brood treatments before the completion of the clutch.

Plot manipulations were achieved by altering the frequencies of the brood size and brood sex ratio treatments differentially among the 12 study plots (Fig. 1, Appendix S2). In that way, we created six different experimental treatments combining a manipulation of nestling density (decreased/increased) and sex ratio (female/balanced/male). Each treatment was randomly assigned to a plot, semi-randomized between years (i.e. each plot received a different treatment in every study year) and occurred in two replicates per year. Female- and male-biased plots were manipulated to *c.* 25% or *c.* 75% male fledglings respectively while balanced plots were manipulated to *c.* 50%, which reflects the natural situation (Fig. 1, Appendix S2). These plot sex ratio biases were at the extreme ends of the natural range of avian offspring population sex ratios yet within the range of avian adult population sex ratios (Donald 2007). Decreased and increased density plots were manipulated to *c.* $\pm 13\%$ of change in the number of nestlings in a plot (Appendix S2). Importantly, it should be realized that variation in brood sex ratio was present between but not within plots (i.e. all the broods of a plot received the same sex ratio treatment), whereas experimental variation in brood size was maintained both within and between plots (i.e. not all the broods of a plot received the same brood size treatment) (Fig. 1). We left the natural breeding densities (number of breeding pairs per plot) unchanged.

FITNESS COMPONENTS AND DATA SELECTION

We analysed variation in the probability of producing a second clutch (given that the first brood was successful) and the adult local survival probability (probability to survive in our study area until the subsequent breeding season). Variation in the fitness benefits of reproduction, that is, the number of recruits per brood, and variation in laying date and clutch size in the next year were also examined. However, because these parameters were not affected by the manipulations, we will not discuss these results further.

Our experiment was performed over 3 years (2005–2006–2007) during which we manipulated the first broods (defined as clutches started within 30 days of the earliest clutch in that year) and monitored breeding traits of the second broods (defined as broods produced by females that were known to have successfully fledged a first brood). Also repeat broods (defined as broods produced by known females of whom the first brood has failed) were monitored. Repeat clutches were left unmanipulated as were nests with a clutch size smaller than three eggs or with very high mortality rate ($> 50\%$) before manipulation. Unmanipulated broods were excluded from further analysis as were broods where only one of the two parents was caught (excluded broods in total represented 35% of all the broods). Nevertheless, nestlings from excluded nests were taken into account in the plot nestling density and sex ratio calculations.

Some adults bred repetitively in two or three of the study years ($n = 260$ individuals). To account for inter-dependency between the measurements, we used one random breeding event for each male and female adult in the analysis ($n = 1012$ individuals and $n = 506$ broods). To check for the robustness of our analyses, all the models were run with different random subsamples that contained other random breeding events of birds breeding in multiple years. The randomization did not affect the results of the adult survival analysis. However, the final models for the probability of producing a second clutch differed in the significance of non-experimental parameters

depending on the random selection made. Therefore, for these parameters, we will present the overall model for one of the subsets chosen randomly, but detailed analyses per year can be found in the Appendix S3.

ANALYSES

Variation in the probability of producing a second clutch and the adult local survival probability were studied using a binomial response model with a logit link function. To account for sources of inter-dependency between measurements, we used generalized linear mixed models (GLMM) distinguishing between variance on 3 or 4 levels: plot, year, nest and/or individual (MLwiN version 2.02; Rasbash *et al.* 2004). Adult local survival was not analysed in mark-recapture models because in our population adult recapture rate if alive is high (0.90 ± 0.05 see Tinbergen & Sanz 2004). It is thus unlikely that experimental effects on adult local survival probability are confounded with heterogeneous detection probability. Here, we define local survival as the product of the true survival and the probability of being captured.

Variation in provisioning behaviour (number of visits per individual per 24 h) was analysed using a Poisson response model with a log link function and with plot, nest and individual fitted as random effects. Year was not included as random effect because we only collected data in a single year (2006).

Carry-over effects of the plot density and sex ratio manipulations on next year breeding density were analysed using a normal response model and with plot and year fitted as random effects.

We tested experimental effects on the fitness components, provisioning rates and next year breeding densities by including the treatments (brood size, 'BS', on the nest level, density, 'plotD', and plot sex ratio, 'plotSR', on the plot level) as factors and the original values (brood size, nestling density and nestling plot sex ratio) as continuous explanatory variables to control for positive correlations between breeding parameters and habitat/individual quality. Laying date (continuous) and parental sex (factor) were included in the models. To control for annual variation in natural ecological settings (e.g. food abundance of temperature), year (factor) and the two- and three-way interactions between year, sex and the experimental variables were also fitted in the models. All continuous covariates were centred on the population mean. Model selection was based on backwards elimination of the non-significant terms in the order of their significance assessed by their Wald statistics. In addition to the best models, we report the most relevant non-significant results retested by reintroducing them into the best model. Means are expressed with standard error. Level of significance was set at $P = 0.05$. The same analyses using the continuous experimental changes of density (' Δ plotD'), plot sex ratio (' Δ plotSR') and brood size (' Δ BS') were also performed and led to the same outcomes.

Results

EFFECTS OF THE MANIPULATIONS

Our manipulation successfully affected the frequencies of brood sizes and brood sex ratios within plots, resulting in significant changes in plot densities and plot sex ratios after manipulation (Fig. 1, Appendix S2 and see details in Nicolaus *et al.* 2009b). Subsequent analyses revealed a significant positive effect of the plot sex ratio manipulation, but

not of the natural plot sex ratio on the breeding densities in the following year. Plots that were male-biased carried higher breeding density the next year than balanced sex ratio or female-biased plots ($+10.3 \pm 3.7\%$ on average; Fig. 2; plot sex ratio treatment: post hoc tests: female vs. male: $\chi^2_{d.f.1} = 5.32$, $P = 0.021$, female vs. balanced: $\chi^2_{d.f.1} = 0.50$, $P = 0.479$, male vs. balanced: $\chi^2_{d.f.1} = 2.64$, $P = 0.104$, overall effect: $\chi^2_{d.f.2} = 5.59$, $P = 0.061$; natural sex ratio: $\chi^2_{d.f.1} = 0.33$, $P = 0.566$). In contrast, the density treatment had no carry-over effect on breeding densities the next year (density treatment: $\chi^2_{d.f.1} = 0.11$, $P = 0.740$). Overall plot breeding densities significantly differed between years and positively correlated to the plot density the previous year (year: $\chi^2_{d.f.2} = 60.22$, $P < 0.001$; natural breeding density: $\chi^2_{d.f.1} = 29.41$, $P < 0.001$). These results showed that the sex ratio but not the density manipulation had a long-lasting effect.

In 2006, data on provisioning behaviour revealed that provisioning rates were significantly lower for both parents rearing small broods than for both parents rearing intermediate or large broods, supporting the hypothesis that parental effort decreased with small brood sizes (Fig. 3a; overall effect: $\chi^2_{d.f.2} = 32.65$, $P < 0.001$ with parental sex, manipulation and original brood size fitted as fixed effects and plot and nest box as random effects, post hoc tests: small vs. intermediate: $\chi^2_{d.f.1} = 10.72$, $P < 0.001$, small vs. large: $\chi^2_{d.f.1} = 31.33$, $P < 0.001$; intermediate vs. large: $\chi^2_{d.f.1} = 2.26$, $P = 0.136$). Brood sex ratio treatment and its interaction with brood size treatment had no effect on provisioning rates (Fig. 3b; sex ratio: $\chi^2_{d.f.2} = 0.17$, $P = 0.917$, interaction: $\chi^2_{d.f.4} = 8.72$, $P = 0.068$). These data show that parental effort was lower for the reduced brood sizes but was not related to brood sex ratio.

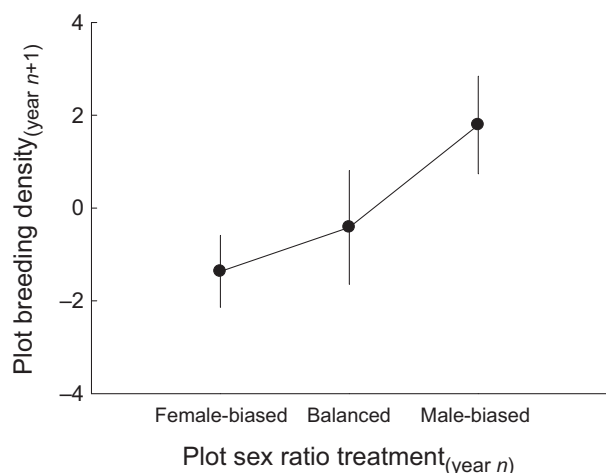


Fig. 2. Carry-over effect of the plot sex ratio manipulation in year n on next year breeding density (number of breeding pairs per plot in year $n + 1$, corrected for years and breeding density in year n) in the Lauwersmeer great tit population. Means are presented with their standard error ($n = 36$ plots).

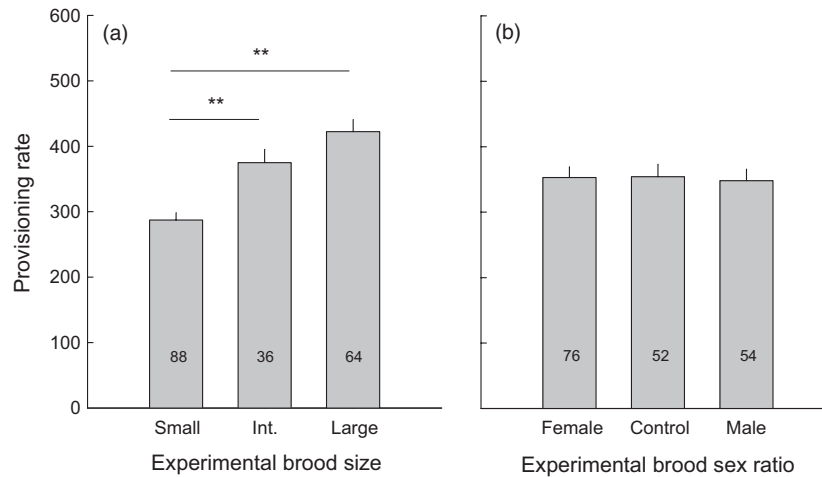


Fig. 3. Effect of the brood size (small/intermediate/large) (a) and brood sex ratio (female/control/male) (b) manipulations on male and female adult provisioning rate (number of visits per 24 h) in a great tit population. Parents provisioned small broods significantly less than intermediate and large broods (post hoc tests indicated with stars; $**P < 0.001$). Brood sex ratio treatment had no significant effect on provisioning rates. Means are presented with standard error (raw data). Sample sizes (number of individual parents) are indicated in the bars.

ADULT LOCAL SURVIVAL PROBABILITY

We found that parents raising small broods in male-biased plots had a higher survival probability than parents raising intermediate or large broods (Table 1, Fig. 4c, post hoc tests: small vs. intermediate: $\chi^2_{d.f.1} = 6.02$, $P = 0.014$, small vs. large: $\chi^2_{d.f.1} = 8.90$, $P = 0.003$, intermediate vs. large: $\chi^2_{d.f.1} = 0.01$, $P = 0.975$). This pattern was absent in plots with balanced sex ratio (Table 1, Fig. 4b). In female-biased plots, there was a non-significant trend for the opposite pattern; namely that parents raising small broods tended to have lower survival probability than parents raising large broods (Table 1, Fig. 4a; post hoc tests: small vs. intermediate: $\chi^2_{d.f.1} = 0.52$, $P = 0.472$, small vs. large:

$\chi^2_{d.f.1} = 3.032$, $P = 0.081$, intermediate vs. large: $\chi^2_{d.f.1} = 0.66$, $P = 0.416$). These experimental effects did not differ between the sexes and were observed in all 3 years (interactions not shown). Overall males and females had similar local survival chance ($\chi^2_{d.f.1} = 0.91$, $P = 0.341$), and adult survival probability was lower in 2005 than in 2006 and 2007 (Table 1 and Appendix S2).

In contrast to one of our expectations, we found no significant interaction between the brood size and the density manipulation (BS \times plotD: $\chi^2_{d.f.2} = 0.30$, $P = 0.862$). The interaction between the plot treatments did not explain additional variance in the survival probability (plotD \times plotSR: $\chi^2_{d.f.2} = 2.20$, $P = 0.331$). Adult survival probability also did not relate to the original plot density or plot sex ratio nor to

Table 1. Final model of analyses examining adult local survival probability in relation to the brood and plot manipulations and some covariates in a great tit population. Experimental brood size ('BS', small/intermediate/large), experimental plot sex ratio ('plot SR', female/balanced/male) and year (2005/2006/2007) are fitted as factors with 'intermediate' brood size', 'balanced' plot sex ratio and '2005' chosen as reference categories. Significant values are shown in bold ($n = 1012$ individuals)

Parameters	Level	B	SE (β)	χ^2	d.f.	P
Intercept		-1.942	0.307			
Plot SR	Year				2	0.449
Female	Year	0.277	0.369			
Male	Year	-0.239	0.431			
BS	Nest			0.24	2	0.885
Small	Nest	-0.110	0.345			
Large	Nest	-0.175	0.355			
Plot SR \times BS	Nest			13.26	4	0.010
Female \times small	Nest	-0.118	0.466			
Female \times large	Nest	0.428	0.474			
Male \times small	Nest	1.027	0.512			
Male \times large	Nest	0.199	0.542			
Year	Year			39.19	2	< 0.001
2006	Year	1.064	0.213			
2007	Year	1.116	0.188			
Random effects	σ^2_{plot}	0.037	0.050	0.56	1	0.455
	σ^2_{year}	0.015	0.060	0.06	1	0.810

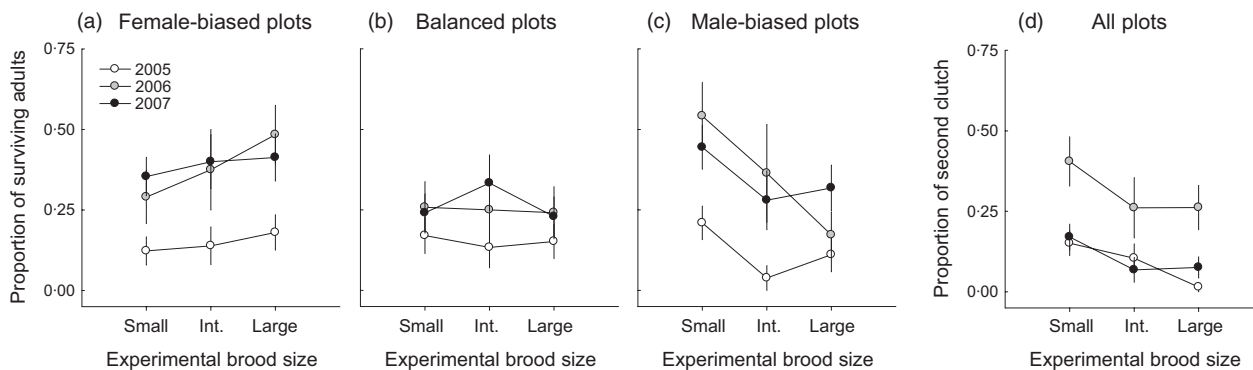


Fig. 4. Effect of the brood size manipulation (small/intermediate/large) on the proportion of surviving adults ($n = 1012$ individuals) in female-biased plots (a), balanced sex ratio plots (b) and male-biased plots (c) and on the proportion of second clutches produced ($n = 506$ nests) (d) in a great tit population. Averages are presented with standard errors for the three study years 2005, 2006 and 2007 (raw data).

the original breeding traits of the adults (not shown). Survival probability tended to be positively correlated with individual probability of producing a second clutch (not shown).

To investigate when the experimental effects on adult survival occurred, we used data on annual mid-winter roosting inspections. We ran the same analysis looking at (i) adult local survival probability until December (early phase of the winter, $n = 1012$ individuals) and (ii) adult local survival probability between December and next year breeding season (April/May) for the subset of adult birds that was alive in December (late phase of the winter, $n = 370$ individuals). We found no experimental effects on adult local survival probability in the early phase of the winter (the interaction between the brood size and the plot sex ratio treatment was not significant: GLMM, experimental effects controlled for sex and years: $\Delta BS \times \Delta \text{plotSR}$: estimate \pm SE = -0.190 ± 0.135 , $\chi^2_{d.f.1} = 1.98$, $P = 0.159$). However, for the subset of birds alive in December, the interaction between the brood size and the plot sex ratio treatment had significant effect on the individual local survival probability in the late phase of the winter (GLMM, experimental effects controlled for sex and years: $\Delta BS \times \Delta \text{plotSR}$: estimate \pm SE = $-0.505 \pm$

0.247 ; $\chi^2_{d.f.1} = 4.18$, $P = 0.041$). This indicates that the experimental effects on adult local survival probability occurred in the late phase of the winter.

THE PROBABILITY OF PRODUCING A SECOND CLUTCH IN THE SAME YEAR

We found a significant negative effect of brood size enlargement on the probability of producing a second clutch (Table 2, Fig. 4d). Unlike the adult local survival probability, the brood size effect was independent of the plot treatments, although there was a trend for a stronger negative effect in increased density plots (plotD \times BS: $\chi^2_{d.f.2} = 5.09$, $P = 0.078$; BS \times plotSR: $\chi^2_{d.f.4} = 2.62$, $P = 0.623$). The interaction between the plot treatments did not explain additional variance in the probability of producing a second clutch (plotD \times plotSR: $\chi^2_{d.f.2} = 2.48$, $P = 0.290$).

The inclusion of natural variation in plot density and plot sex ratio into the model revealed that the production of second clutches was positively correlated with the natural plot sex ratio (Table 2). The production of second clutches correlated negatively with the natural plot density and with the season (Table 2). Birds produced more second clutches in 2006

Table 2. Final model of analyzes examining the probability of producing a second clutch in relation to the brood and plot manipulations and some covariates in a great tit population. Experimental brood size ('BS', small/intermediate/large) and year (2005/2006/2007) are fitted as factors with 'intermediate' brood size, and '2005' chosen as reference categories. Other covariates are fitted as continuous variables. Significant values are indicated in bold ($n = 506$ broods)

Parameters	Level	β	SE (β)	χ^2	d.f.	P
Intercept		-2.783	0.523			
Natural plot density	Year	-0.015	0.008	4.02	1	0.045
Natural plot sex ratio	Year	15.505	6.291	6.07	1	0.014
BS	Nest			9.93	2	0.007
Small	Nest	0.666	0.391			
Large	Nest	-0.546	0.468			
Date (April days)	Nest	-0.202	0.045	20.39	1	< 0.001
Year	Year			19.17	2	< 0.001
2006	Year	1.916	0.606			
2007	Year	-0.802	0.616			
Random effects	σ^2_{plot}	0.143	0.349	0.17	1	0.682
	σ^2_{year}	0.794	0.499	2.54	1	0.110

than in 2005 and 2007 (Table 2 and Appendix S2). Patterns among different random subsets of the data were similar in their experimental effects to the analysis presented (Appendix S3). The analyses per year did not reveal a significant effect of the brood size manipulation. This was probably due to sample bias, because most of the second clutches were produced in 2006 (Appendix S2).

Discussion

The aim of our study was to investigate whether the outcome of reproductive trade-offs depends on the number and sex ratio of fledglings in the local environment. We showed that sex ratio manipulation on the plot level had long-lasting effects on local densities. Surprisingly, the manipulation did not affect the benefits of reproduction, that is, the number of recruits per brood. Instead, we showed that, consistently over the 3 years of the study, local sex ratio affected the survival cost of reproduction. We also found a fecundity cost of reproduction in the same year, but this did not depend on the plot density or sex ratio. This important result implies that the survival cost of reproduction and thus selection on reproductive rates depends on the local social environment.

SURVIVAL COST OF REPRODUCTION AND LOCAL SEX RATIO

We found that male-biased plot treatment had a significant positive effect on local breeding density the next year and that all parents caring for experimentally reduced broods decreased their provisioning rate. However, only parents caring for small broods in male-biased plots survived better. These findings imply that raising a small brood has long-lasting effects on some phenotypic traits of the parents and that this can increase their survival chances in male-biased environment.

Experimental studies have shown that brood manipulations often affect parental feeding activity and energy expenditure (Sanz & Tinbergen 1999; Nilsson 2002), presumably to the detriment of individual 'state' (e.g. reduced body mass, Nur 1984; reduced immune response, Pap & Markus 2003; or delayed moult, Svensson & Nilsson 1997). These effects on state are important because they give rise to variation in individual phenotypic quality that may affect individual ability to cope with social (Gosler & Carruthers 1999; Lindström, Hasselquist & Wikelski 2005; van Oort *et al.* 2007), or physiological challenges (Gustafsson *et al.* 1994; Stjernman, Råberg & Nilsson 2004; Ardia 2005). The fact that high proportions of males in the environment increased breeding densities the following year and also increased the survival cost of reproduction suggests that increased local densities may have led to a deterioration in habitat quality. This might have occurred through increased competition or through increased predation or disease risks (Newton 1998). It also suggests that a high phenotypic quality enabled individuals to better survive these harsh conditions. This may be especially true at the end of the winter when resources have

decreased, and when competition within- and between-sexes (e.g. for food) drastically increases (Drent 1983, 1984), or when males express intense territorial behaviour accompanied with many agonistic interactions (Drent 1983; Nicolaus *et al.* 2009a). Thus, the survival cost of reproduction is most likely the product of an interaction between parental phenotypic quality after reproduction and individual social environment. In this study, provisioning rates showed some levelling off in the number of visits to the nest with brood size, which may explain why parents raising intermediate and large broods had similar survival probability.

In contrast to the increased survival of parents raising small broods in male-biased plots, we found a trend for a positive effect of parental effort on adult survival in female-biased plots. A lower fledgling density in female-biased plots (Michler 2010) in combination with an increased dispersal of nestling from enlarged broods (Tinbergen 2005) may have relieved parents raising large broods from post-fledging parental care (Verhulst & Hut 1996) and/or may have reduced the level of within-family competition in environments where mortality risks were potentially lower (decreased competition or predation/disease risks; Newton 1998). Parents may also have invested less in female nestlings from enlarged broods that have low fitness prospects. These factors, in combination with a lower probability of producing a second brood, may have translated into a higher survival probability for parents with large broods.

The absence of sex-specific experimental effects on adult survival suggests that plot sex ratio manipulation did not affect the level of intra- or inter-sexual competition. Juveniles may have competed with adults for non-sex-specific resources such as food or shelter. Therefore, the overall number of juveniles may have affected the level of intraspecific competition and the adult sexes in a similar way. Alternatively, the manipulation may have not affected competition per se but rather has affected other ecological aspects of the environment such as predation or disease risks that may affect the sexes similarly. Another possibility is that adult males and females have been affected by the manipulation through different pathways that had similar outcomes.

We believe that the effect of experimental sex ratio on brood-related fitness occurred at the plot level and was not an individual brood effect. This is because the addition of brood sex ratio in the models did not explain additional variation (tested but not shown). Additionally, we could not detect any fitness effects of brood sex ratio during the nestling period (effect on nestling performance, Nicolaus *et al.* 2009b), or any effects on provisioning rates (this study). However, to exclude any alternative possibility, brood sex ratio should be manipulated independently from the experimental plot sex ratio.

FECUNDITY COST OF REPRODUCTION

A fecundity cost of reproduction in the same year was found independently of the plot manipulation. As found in many studies, adults raising an enlarged brood were less likely to

produce a second clutch in the same year than adults raising a reduced brood (reviewed in Parejo & Danchin 2006). We thus infer that overall selection on brood size in this study was directional towards small broods. Presumably, adults with experimental large broods became of too low condition to successfully raise a second brood or they have traded-off the duration of the post-fledging parental care of the first brood with the production of a second brood (Verhulst, Tinbergen & Daan 1997). The occurrence of a second clutch was also reduced in natural high-density plots and natural male-biased plots. Consistent with classical negative density-dependent patterns, breeding birds may adaptively adjust their reproductive decisions to the expected level of intraspecific competition for local resources during or after the nestling phase (Nicolaus *et al.* 2009a). The presence of good-quality habitat or good-quality breeders may also result in the production of more of the philopatric sex (males; Doligez *et al.* 2008) and may lead to the production of more second clutches.

COSTS OF REPRODUCTION IN OTHER NEST-BOX POPULATIONS

We propose that costs of reproduction depend on the social context in which they are estimated. In our study, social effects on adult survival could probably be detected because we manipulated local fledging density and sex ratio. This suggests that brood size manipulations alone may not be the most appropriate way to measure the costs of reproduction. This is because a change of brood size affects the number and the condition of the fledglings and thus the phenotypic quality of local competitors (via growth, Nicolaus *et al.* 2009b). By enlarging broods, we not only increase the local density of fledglings but we also decrease the competitive ability of these individuals. As a consequence, the local level of intraspecific competition may change and subsequently affect the costs of reproduction for the local parents. This may be the reason why we found an effect of the experimental local sex ratio (not related to the condition of the local fledglings), but not of experimental density (related to the condition of the local fledglings). It may also explain why the costs of reproduction are not consistently detected in other populations.

Conclusions

Our study supports the hypothesis that local sex ratio effects mediate the costs of reproduction and thus may affect optimal clutch size. The implication is that the fitness consequences of clutch size depend not only on the choice of individual parents, but also on the choices of other breeding pairs in the population. Most likely, the relative phenotypic quality of other members in the population is decisive in determining the outcome of competitive interactions and subsequent survival. Alternatively, other indirect social effects such as increased predation risk or the transmission of diseases or parasites may play a role. These findings highlight the importance of incorporating social effects of the

environment in future studies on the physiological costs of reproduction.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Collection of provisioning data.

Appendix S2. Overview of the plot manipulations and breeding parameters of the Lauwersmeer great tit population in the Netherlands.

Appendix S3. Annual variation in the probability of producing a second clutch.

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