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Local offspring density and sex ratio affect sex allocation in the great tit

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Supplementary data

The following supplementary data is available for this article online.

How experimental treatments were assigned to plots, broods and nestlings

Each plot treatment-combination was semi-randomly allocated to plots each year (not allowing for a plot to have the same combination in consecutive years). For this we assigned plots at the start of the breeding season to two groups of early and later plots based on average start of laying date per plot. The replicates of plot treatments were then distributed within these groups such that every treatment was assigned once to an early and a later plot. By doing so the degree of between plot synchronization should have been about equally distributed over the treatment groups.

For the brood sex ratio treatment all broods within a plot were assigned the same treatment (female-biased broods in female-biased plots etc). We aimed at manipulating female-biased broods to an average sex ratio of 25 % males, control brood to 50% males and male-biased broods to 75 % males. Variation around these sex ratios occurred within each brood sex ratio treatment because brood sizes varied and because some broods had not enough or too many nestlings of a given sex. We always kept at least one nestling of the opposite sex in each brood. To assign the brood size treatment, all first broods within a plot were listed according to their expected hatching date. We then distributed the three brood size treatment categories (reduced control and enlarged) in the required proportion for the plot density treatment (e.g. 20% reduced broods, 20% control broods and 60% increased broods in high density plots) such that the brood treatments were equally distributed over the expected hatching dates. When a brood was abandoned or died before the manipulation at day 6, it was cancelled from the experimental broods list. Consequently, we adjusted the treatments of broods that had not yet been manipulated up to that date according to the proportion of brood treatments required for the plot treatment and equal distribution over date. We were blind to all original brood characteristics (clutch size, brood size, brood sex ratio etc.), except for the expected hatching date, when we assigned the brood treatment.

All brood members were treated as equivalent reproductive units and we selected them at random for manipulation. To select nestlings for swapping we use the clip numbers from the marking on day 2. At day 5 (one day before swapping) we checked which nestlings were still present and alive in the brood. For the swapping planning (made on day 5) we chose nestlings of the sex needed for the manipulation assigned to the brood (e.g. usually for a brood with a female-biased treatment male nestling were removed and females added). Generally we started with chick nr 1 to assign nestlings to the brood they had to be transferred to. Although in this way the low clip numbers in a brood were more likely to be transferred to another brood, this still follows a random procedure as nestlings on day 2 were selected in a random manner for nail clipping, following successive numbering from 1 onwards (up to the number of nestlings in the brood). For all experimental broods at least one nestling was exchanged such that each brood contained own and foster nestlings. When individual nestlings within a brood were found dead on the day of swapping, we adjusted the swapping planning (the sex of the nestling and/or number of nestlings to be transferred) with the least possible deviation from the original plan.

Legends

Fig. S1: Experimental plot manipulation of density and sex ratio of nestling great tits for the years 2005-2007. Experimental changes in (A) the sex ratio of nestlings per plot for the three sex ratio treatment categories and (B) the number of nestlings per plot for the two density treatment categories. Changes are calculated by subtracting the natural number and natural sex ratio of young per plot at day 6 from the final experimental number and final experimental sex ratio of young per plot at day 6. Averages are presented with standard errors (raw data).

Fig. S2: Frequency distribution of brood sex ratios before manipulation (A) and after manipulation (B) on day 6 for female-biased plots (light grey bars) control plots (dark grey) and male-biased plots (black). Brood sex ratios were grouped for 0-0.1, 0.1-0.2 and so forth.

Fig. S3: Frequency distribution of brood sizes before manipulation (A) and after manipulation (B) on day 6 for low density plots (light grey bars) and high density plots (dark grey).

Fig. S4: Natural (unmanipulated) nestling density at day 6 per plot for the years 2005 (filled circles), 2006 (open triangles) and 2007 (filled squares) for plots that received a low or a high density treatment.

Fig. S5: Observed sex ratio of juveniles averaged per experimental sex ratio treatment at fledging (based on young that were known to have fledged) and per monthly observation period. Observed sex ratio after fledging was calculated from observations of successfully fledged colour ringed juveniles in the post fledging period in the whole study area in 2005 (upper graph, 1866 sightings of 903 juveniles) and 2006 (lower graph, 1345 sightings of 663 juveniles). Via coordinates each sighting of an individual young was associated to the nearest nest box plot (first sighting in each month) to calculate the observed sex ratio in and around each nest box plot per month from June till October. Averages are shown per experimental plot sex ratio treatment where black squares are plots that had a male-biased sex ratio treatment, grey triangles are control sex ratio plots and open circles are plots with a female-biased sex ratio treatment. Standard errors are based on raw data.

Fig. S6: Observed number of juveniles per plot (log10-transformed) averaged per experimental density treatment at fledging (based on young that were known to have fledged) and per monthly observation period. Observed number after fledging was calculated from observation of successfully fledged colour ringed juveniles in the post fledging period in the whole study area in 2005 (upper graph, 1866 sightings of 903 juveniles) and 2006 (lower graph, 1345 sightings of 663 juveniles). Via coordinates each sighting of an individual young was associated to the nearest nest box plot (first sighting in each month) to calculate the observed number in and around each nest box plot per month from June till October. Averages are shown per experimental density treatment where light grey bars refer to plots that had a low density treatment and dark grey bars are plots with a high density treatment

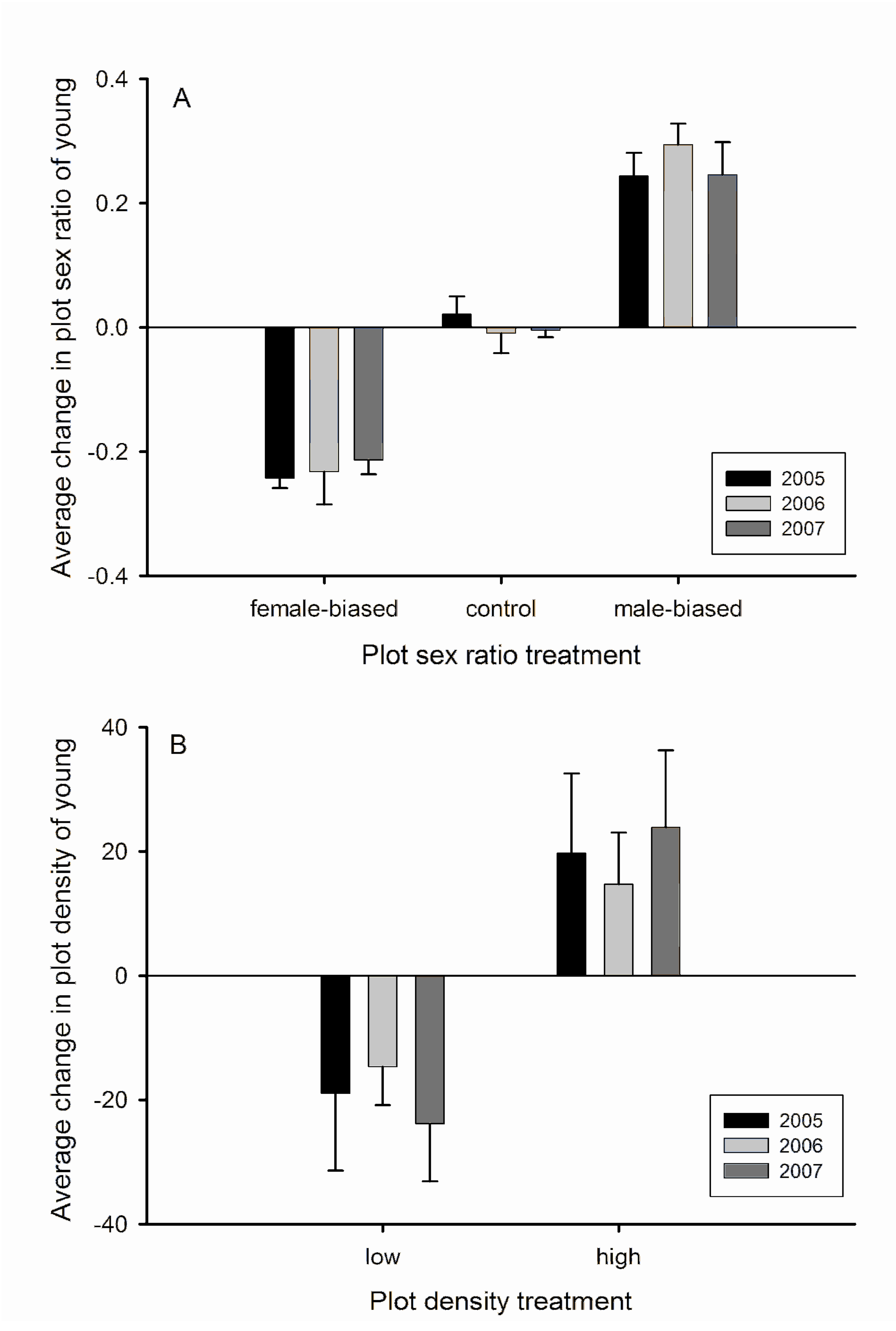
A
Average change in plot sex ratio of young
0.4
0.2
0.0
-0.2
-0.4
female-biased
control
male-biased
Plot sex ratio treatment
2005
2006
2007
B
Average change in plot density of young
40
20
0
-20
-40
low
high
Plot density treatment
2005
2006
2007

Fig. S1

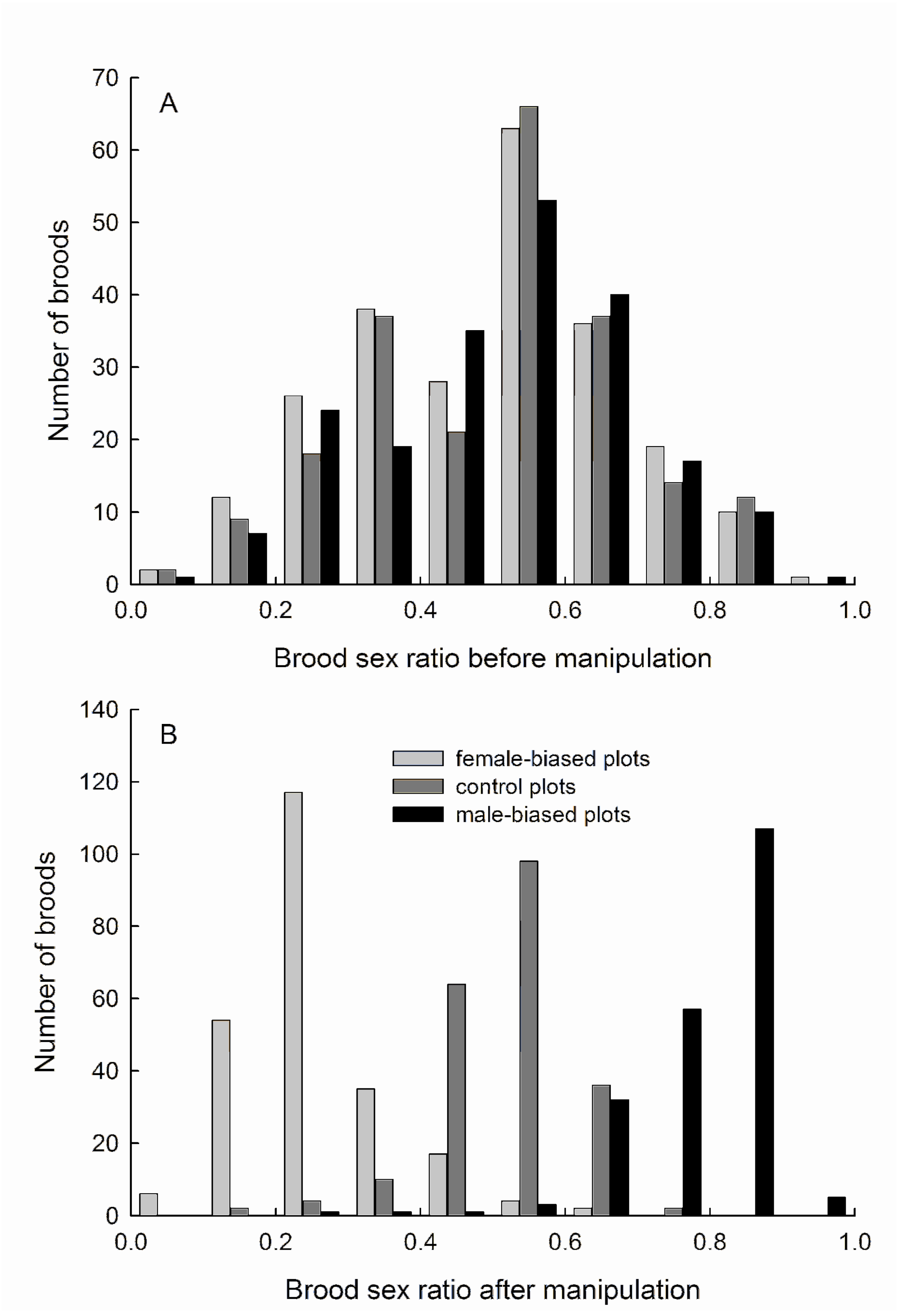
A
70
60
50
40
30
20
10
0
Number of broods
0.0
0.2
0.4
0.6
0.8
1.0
Brood sex ratio before manipulation
B
140
120
100
80
60
40
20
0
Number of broods
female-biased plots
control plots
male-biased plots
0.0
0.2
0.4
0.6
0.8
1.0
Brood sex ratio after manipulation

Fig. S2

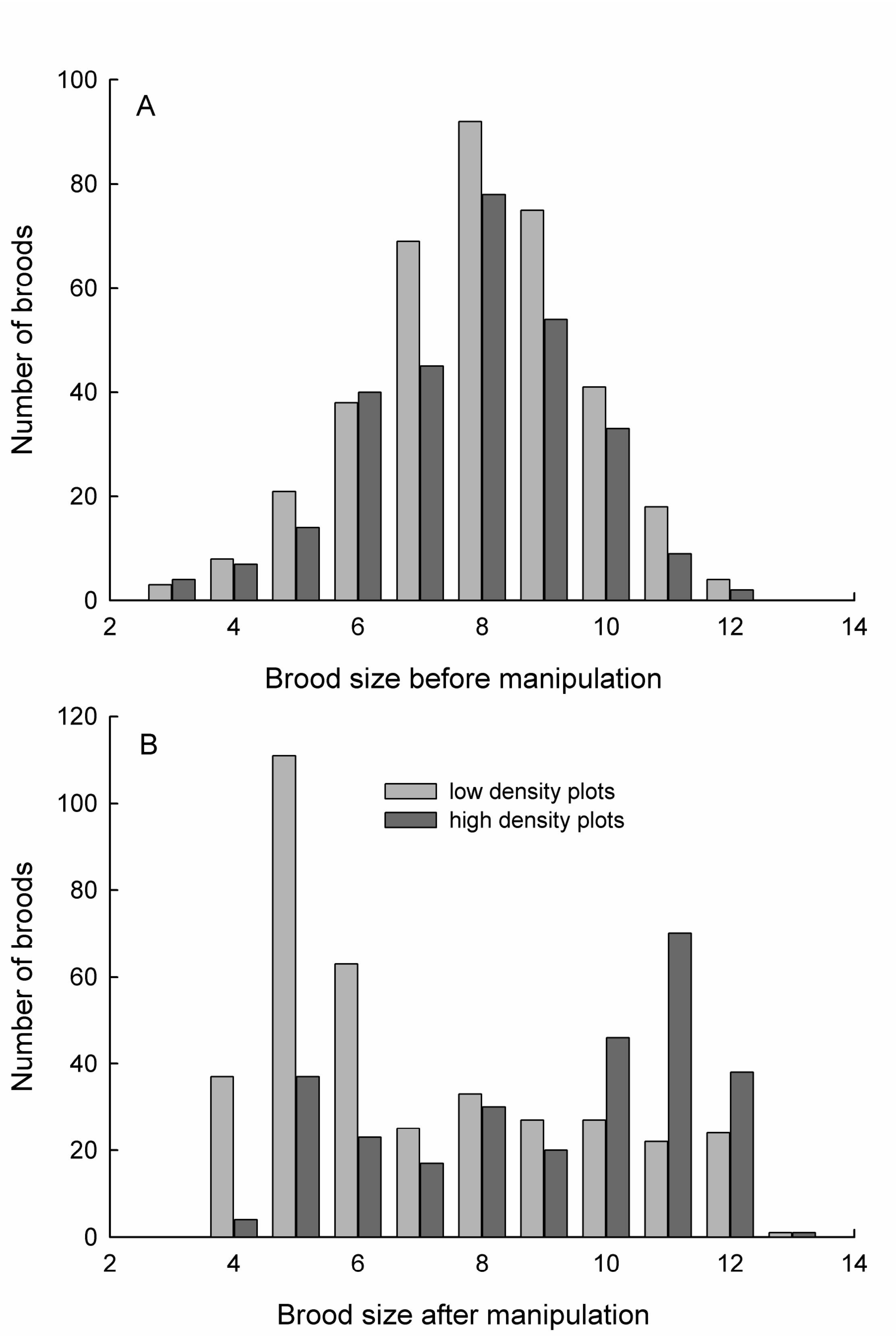
A
Number of broods
0
20
40
60
80
100
2
4
6
8
10
12
14
Brood size before manipulation
B
low density plots
high density plots
Number of broods
0
20
40
60
80
100
120
2
4
6
8
10
12
14
Brood size after manipulation

Fig. S3

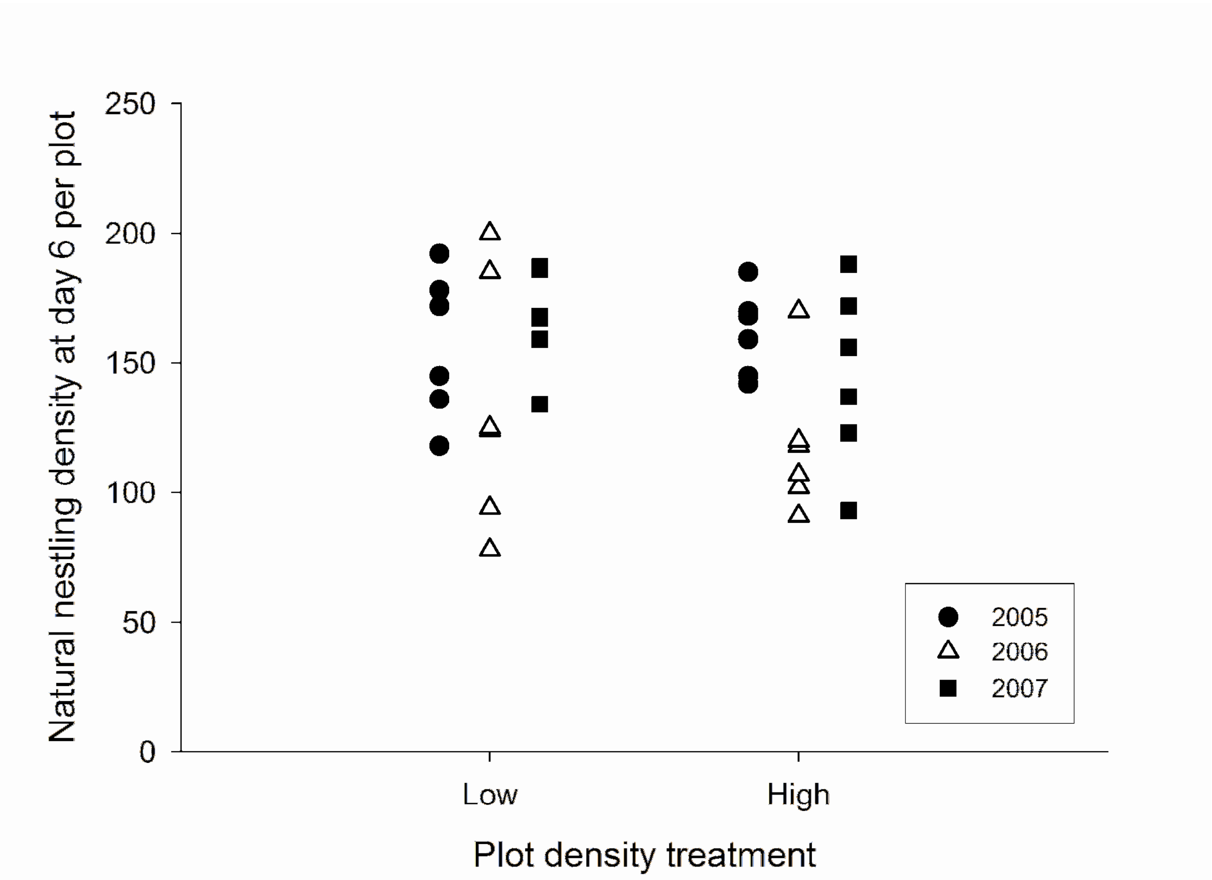
Natural nestling density at day 6 per plot
250
200
150
100
50
0
Low
High
Plot density treatment
● 2005
△ 2006
■ 2007

Fig. S4

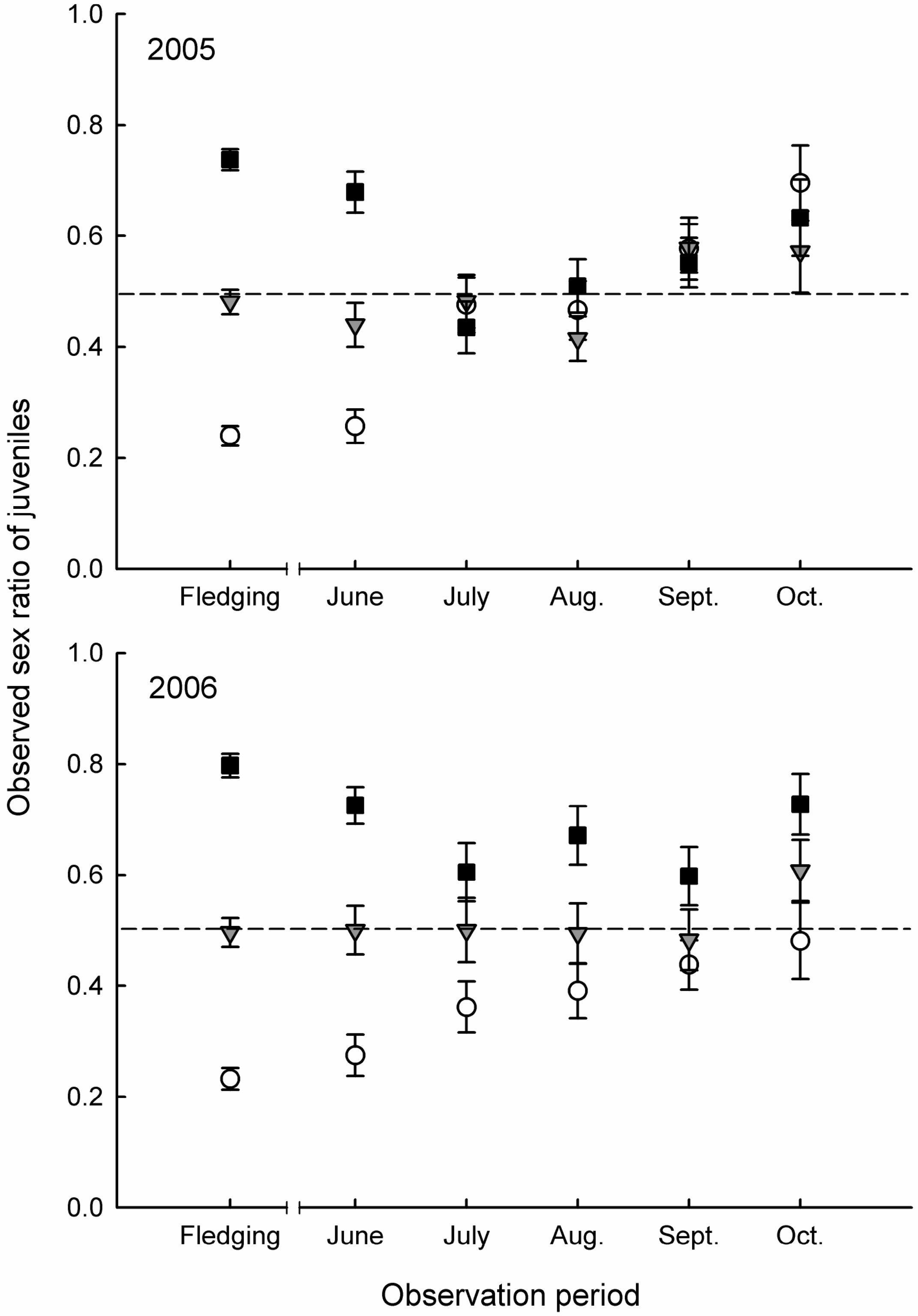
1.0
0.8
0.6
0.4
0.2
0.0
2005
2006
Observed sex ratio of juveniles
Fledging
June
July
Aug.
Sept.
Oct.
Observation period

Fig. S5

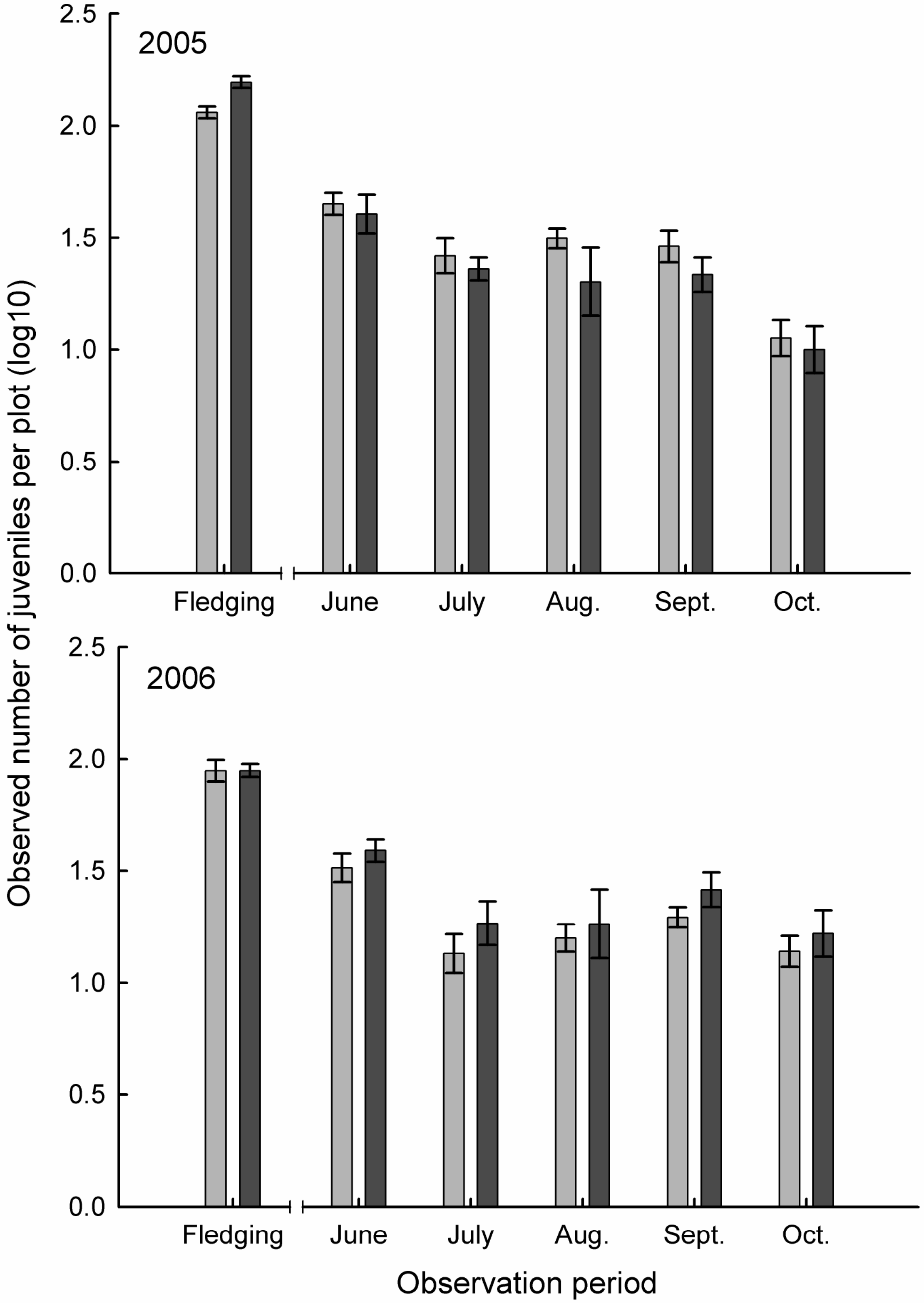
2005
2006
2.5
2.0
1.5
1.0
0.5
0.0
Observed number of juveniles per plot (log10)
Fledging
June
July
Aug.
Sept.
Oct.
Observation period

Fig. S6

Table S1: Plot sex ratio (left columns) and plot density (right columns) of nestlings at nestling age 6, 14 and at fledging per sex ratio and density treatment category. Differences between treatment categories were tested using Kruskal-Wallis for the plot sex ratio treatment and independent t-test for the density treatment. Statistically significant p-values (at the 0.05 level) are given in bold.

	Female-biased plot sex ratio	Control plot sex ratio	Male-biased plot sex ratio	Kruskal-Wallis test	High density treatment	Low density treatment	Independent t-test
	mean±SD, N			χ^2, df, p	mean±SD, N		t, df, p
Nestlings day 6	0.24±0.01, 12	0.49±0.01, 12	0.75±0.01, 12	16.0, 2, **< 0.001**	156.0±35.2, 18	127.1±37.0, 18	-2.52, 34, **0.02**
Nestlings day 14	0.23±0.02, 12	0.49±0.02, 12	0.77±0.03, 12	24.0, 2, **< 0.001**	126.0±36.1, 18	107.1±26.2, 18	-1.80, 34, 0.08
Fledglings	0.23±0.02, 12	0.49±0.03, 12	0.76±0.03, 12	24.0, 2, **< 0.001**	118.3±36.3, 18	101.1±23.9, 18	-1.68. 34, 0.10

Table S2: Correlation between year t and year t+1 for plot natural nestling sex ratios (day 6), natural nestling densities (day 6) and plot breeding pair densities (number of incubating first broods) and correlation between plot experimental nestling density (year t) and breeding pair density (year t+1). For the later we also show results controlling for natural nestling density in year t (controlled experimental density). Between year relation over all years (2005-2008) for plot traits was analysed in a GLM with the plot trait in year t+1 as dependent variable and the plot trait in year t as explanatory variable controlling for year. Correlation coefficients for each year comparison separately are given for Spearman rank correlations (R_s) and Pearson's correlations (R_c). P-values in bold indicate significance at the 5% level.

	Overall between year relation (controlled for year)	2005-2006	2006-2007	2007-2008
Plot natural nestling sex ratios (day 6)	$\chi^2 = 1.51$ df = 1 p = 0.219	$R_s = 0.35$ n = 12 p = 0.265	$R_s = -0.54$ n = 12 p = 0.071	$R_s = -0.35$ n = 12 p = 0.313
Plot natural nestling densities (day 6)	$\chi^2 = 3.02$ df = 1 p = 0.082	$R_c = 0.30$ n = 12 p = 0.346	$R_c = 0.49$ n = 12 p = 0.104	$R_c = 0.05$ n = 12 p = 0.864
Plot breeding pair densities	$\chi^2 = 17.41$ df = 1 **p < 0.001**	$R_c = 0.823$ n = 12 **p = 0.001**	$R_c = 0.47$ n = 12 p = 0.123	$R_c = 0.71$ n = 12 **p = 0.010**
Plot natural nestling density (t) with plot breeding pair density(t+1)	$\chi^2 = 10.75$ df = 1 **p = 0.001**	$R_c = 0.30$ n = 12 p = 0.38	$R_c = 0.65$ n = 12 **p = 0.022**	$R_c = 0.43$ n = 12 p = 0.166
Plot controlled experimental nestling density (t) with plot breeding pair density (t+1)	$\chi^2 = 0.03$ df = 1 p = 0.853	$R_c = -0.22$ n = 12 p = 0.493	$R_c = 0.13$ n = 12 p = 0.693	$R_c = 0.13$ n = 12 p = 0.677
Plot uncontrolled experimental nestling density (t) with plot breeding pair density (t+1)	$\chi^2 = 6.88$ df = 1 **p = 0.009**	$R_c = 0.05$ n = 12 p = 0.882	$R_c = 0.58$ n = 12 **p = 0.050**	$R_c = 0.49$ n = 12 p = 0.104