



Effects of predator call playbacks on reproductive success and extrapair paternity in blue tits

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An increase in the perceived risk of predation triggers many behavioural changes in prey species, which can have consequences for their reproductive success. Perceived predation risk may also influence investment in extrapair activities and, as a result, the frequency of extrapair paternity (EPP), but this possibility remains largely untested. Here we report on a study of a small passerine bird, the blue tit, *Cyanistes caeruleus*, in which we experimentally manipulated perceived predation risk by intermittently broadcasting predator calls throughout the breeding season. We found no evidence that the treatment affected two behavioural indices of extrapair activity (extrapair visits and the time of emergence from the nestbox in the morning during the fertile period) or the rate of EPP itself. The treatment also had no significant effect on clutch size, hatching success or most reproductive behaviours. However, nests in the predator playback treatment produced more fledglings, which was mainly due to a lower frequency of complete brood mortality. We discuss potential explanations for this finding, as well as for the lack of evidence for other effects of the predator playback treatment on blue tit reproductive behaviour. Several measures of reproductive performance suggest that the year in which the experiment took place was an unusually poor one and further work is therefore needed to assess the generality of our findings.

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The most obvious effects that predators have on prey animals are those related to direct killing, but there is growing evidence that predators also have substantial nonconsumptive effects (Creel & Christianson, 2008; Cresswell, 2008; Lima, 2009). For instance, the perceived risk of predation can have large effects on the reproductive behaviour of prey species (sometimes referred to as 'fear effects'). Experimental studies have demonstrated that perceived predation risk affects various aspects of reproductive investment in songbirds, including the number of eggs produced, incubation effort and nestling provisioning (Basso & Richner, 2015; Fontaine & Martin, 2006; Ghalambor, Peluc, & Martin, 2013; Ghalambor & Martin, 2002; Julliard, McCleery, Clobert, & Perrins, 1997; LaManna & Martin, 2016; Zanette, White, Allen, & Clinchy, 2011). Such nonconsumptive effects of perceived predation risk can have subsequent negative effects on the number of offspring produced by prey species and thus have important demographic

consequences (Hua, Sieving, Fletcher, & Wright, 2014; LaManna & Martin, 2016; Sheriff, Krebs, & Boonstra, 2009; Zanette et al., 2011).

There is considerable variation in the degree to which individuals of different species or populations respond to increased perceived predation risk, even under identical experimental conditions. In songbirds, the degree to which individuals reduce investment differs between species, and some species show no response or even increased investment in response to an increase in perceived predation risk (Fontaine & Martin, 2006; Ghalambor et al., 2013; LaManna & Martin, 2016). This is partly driven by variation between species in the probability of repeat breeding, with a low residual reproductive value (i.e. a low probability of future breeding) being associated with reduced responsiveness to changes in predation risk (Clark, 1994; Ghalambor & Martin, 2001, 2002; LaManna & Martin, 2016). Species subjected to high nest predation rates also show stronger responses to changes in predation risk (Eggers, Griesser, Nystrand, & Ekman, 2006; Fontaine & Martin, 2006; Ghalambor et al., 2013; Martin & Briskie, 2009). Indeed, a study of 10 songbird species reported that all species reduced nestling provisioning in response to a predator presentation, but that provisioning rates decreased more in species that had a greater risk of nest predation (Ghalambor et al., 2013).

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Perceived predation risk may also influence investment in mating behaviour and extrapair activities in songbirds and thus lead to changes in the frequency of extrapair paternity (EPP). However, effects on EPP and related behaviours remain largely untested (Abbey-Lee et al., 2018). EPP is common in birds, but rates vary substantially within and between species (Griffith, Owens, & Thuman, 2002; Westneat & Stewart, 2003). This has attracted considerable interest from evolutionary biologists, because EPP may increase male–male competition and the importance of female choice and therefore increase the potential for sexual selection in socially monogamous species (Griffith et al., 2002; Schlicht & Kempenaers, 2013; Webster, Pruett-Jones, Westneat, & Arnold, 1995). Most work has focused on ultimate explanations for this variation, but little is known about the factors that may limit the occurrence of EPP. Actual or perceived predation risk may be one such factor. If this is the case, perceived predation risk may not only have demographic effects, as has been demonstrated earlier, but also have consequences for sexual selection.

Patterns of EPP should be particularly affected by the perceived risk of predation, because the behaviours performed in search of extrapair matings are likely to increase the probability of encounters with predators (Alcock, 1995; Jennions & Petrie, 1997; Sih, 1987, 1992). For instance, extrapair matings typically involve conspicuous advertisement to attract extrapair mates, such as singing or visual displays. Such conspicuous displays could attract not only potential mates, but also predators (Magnhagen, 1991; Hughes et al., 2012). Moreover, given that most extrapair fathers are breeding males, either the male or the female must leave its territory in search of an extrapair mate. Previous studies have demonstrated that these extraterritorial forays lead to increased risk of predation and associated stress (Ridley, Raihani, & Nelson-Flower, 2008; Young & Monfort, 2009). Individuals must thus trade the benefits of advertising and pursuing extrapair activities against the cost of being more susceptible to predation. As a consequence, individuals are expected to reduce their investment in extrapair behaviour in response to an increase in the risk of predation (Gibson & Langen, 1996; Westneat, Sherman, & Morton, 1990).

We tested the effects of perceived predation risk on breeding behaviour and EPP in blue tits, *Cyanistes caeruleus*, by intermittently broadcasting predator calls throughout the breeding period. Blue tits have a fast life history: interannual mortality is high and breeding pairs produce only one brood per year in our study population, such that many birds breed only once in their life (Santema & Kempenaers, 2018). Because blue tits nest almost exclusively in artificial boxes in which they are protected from predators, nestling mortality through predation is virtually nonexistent, at least in our population. However, parents are at risk of predation from sparrowhawks, *Accipiter nisus*, and other aerial predators (Perrins, 1979; Santema & Kempenaers, 2018). Variation in reproductive success is substantial, and may arise from differences in clutch size, hatching success and fledging success (Perrins, 1979; Santema & Kempenaers, 2018). Moreover, EPP is common, with about half (31–65%) of the broods containing extrapair young (Delhey, Johnsen, Peters, Andersson, & Kempenaers, 2003; Kempenaers et al., 1992, 1997; Schlicht, Valcu, & Kempenaers, 2015a). This moderate level of EPP, in combination with the high breeding density at our study site (70–140 breeding pairs annually), makes it a particularly suitable system for this study.

We also examined the behavioural processes that may mediate the effects of perceived predation risk on breeding success and EPP. We measured two key parental care behaviours: time spent incubating and provisioning rate. In previous studies on other passerine birds, both behaviours were affected by experimentally increasing perceived predation risk (Fontaine & Martin, 2006; Ghalambor et al., 2013; Zanette et al., 2011). We also investigated two

behaviours associated with EPP in blue tits: extrabox visits and morning emergence time during the fertile period. In blue tits, both males and females visit territories and nestboxes occupied by other breeding pairs immediately prior to and during the egg-laying period (Kempenaers et al., 1992; Schlicht, Valcu, & Kempenaers, 2015b), and males often sire extrapair offspring with females whose box they visited (Schlicht et al., 2015b). Although a direct link between emergence time and EPP has not been shown (Schlicht, Valcu, Loes, Girg, & Kempenaers, 2014), extrapair copulations typically occur early in the morning (Kempenaers, 1994) and males that start singing the earliest are more likely to sire extrapair offspring (Poesel, Kunc, Foerster, Johnsen, & Kempenaers, 2006; see also ; Kempenaers, Borgström, Loes, Schlicht, & Valcu, 2010). Morning emergence time and extrabox visits may therefore be indices for extrapair behaviour in blue tits. We used an automatic nest visit monitoring system based on radio frequency identification (RFID) technology to obtain comprehensive data on emergence time and extrabox visits, as well as on the amount of time spent incubating and provisioning rate.

In this study, we tested two general hypotheses about the effects of increased perceived predation risk. First, we tested whether increasing the perceived risk of predation by playing back predator calls leads to reduced investment in behaviour related to seeking extrapair copulations (later emergence from the nestbox and fewer extrabox visits) and, as a consequence, lower levels of EPP. Second, we tested whether an increase in perceived predation risk leads to reduced investment in reproduction (lower clutch size, less incubation and lower frequency of nestling provisioning) and, consequently, to reduced offspring condition (shorter tarsi and lower body mass) and lower reproductive success (lower hatching and fledging success).

METHODS

Manipulation of Perceived Predation Risk

We performed the experiment in a population of blue tits in the Westerholz forest near Landsberg am Lech, Southern Germany (48°08'26"N, 10°53'29"E), during the 2016 breeding season. The ca. 40 ha study area consists of mixed deciduous forest dominated by mature oak trees and contains 277 nestboxes of which 114 were occupied by blue tits. We divided the study site into a predator playback plot, occupying the northern portion of the site, and a similarly sized nonpredator playback plot, occupying the southern portion of the site (Appendix Fig. A1). In each plot, we installed 25 Foxpro Shockwave speakers (Foxpro Inc., Lewiston, PA, U.S.A., www.gofoxpro.com), corresponding to roughly one speaker for every two blue tit territories. In the predator playback (north) plot, we increased perceived predation risk by intermittently broadcasting calls of predators, whereas in the nonpredator playback (south) plot we broadcast calls of nonpredatory birds (see details below). Between the two plots, we maintained a buffer zone of 80 m (roughly one territory diameter) to prevent birds in one plot from being exposed to sounds from the other plot. The treatment started on 30 March 2016, when the first nest was completed and 10 days before the first egg in the population was laid, and continued until 6 June 2016, when all nestlings had fledged.

We used one large plot for each treatment rather than multiple smaller plots, for two reasons. First, our primary aim was examining how elevated perceived predation risk affects extrapair behaviour and EPP. Extrapair behaviour involves interactions between individuals from different territories, requiring areas at a larger geographical scale in which all individuals are exposed to the same treatment. Second, multiple smaller plots would have led to a substantial reduction in the total number of experimental and

control nests, because a greater total buffer area would be required. Although the lack of replicates presents a clear limitation, data collected in previous, nonplayback years, using the same protocols, enabled us to test for consistent differences between the northern and southern portions of the study site. Depending on the variable, data from 1–6 previous years were available. We could therefore not only test whether there was a difference between the north (predator) and south (nonpredator) plots during the playback year (2016), but also whether this difference between the north and south plots was absent in previous, nonplayback years.

Calls were broadcast following the protocol used in studies that have shown substantial effects on behaviour and measures of reproductive success in other songbirds (Hua et al., 2014; LaManna & Martin, 2016; Zanette et al., 2011). These protocols were designed to increase the level of perceived predation risk while minimizing the risk of habituation. We broadcast recordings from 2 h before sunrise to 1 h after sunset at a ratio of 1:1.5 sound to silence (e.g. a 60 s recording was followed by 90 s of silence, see Zanette et al., 2011). We broadcast calls of nocturnal species before sunrise and after sunset, calls of both nocturnal and diurnal species in the first hour after sunrise and calls of diurnal species during the rest of the day. Recordings were played back at a volume of 85 dB at 1 m from the speaker. We placed the speakers about 8 m from active blue tit nests, about 1.5 m high and facing the nestbox (LaManna & Martin, 2016). To reduce the risk of habituation, we moved speakers between nestboxes every second day, such that each nest was exposed to the playbacks on a 2-day on/2-day off schedule. Only half of the nests therefore had a speaker next to it at any given time, but on days when a nest had no speaker located next to it, calls were still audible from speakers at surrounding nests (albeit at a lower volume). Following clutch completion, we moved speakers between nestboxes every fourth day (4-day on/4-day off schedule; Zanette et al., 2011; Abbey-Lee et al., 2018). Moving speakers involved walking each plot in a systematic way for 2 h, and during this time we noted any observation of avian predators (total observation time: 100 h per plot).

For the predator playback treatment, we used six nocturnal predators and 12 diurnal predator species that occur in southern Germany (Appendix Table A1). All these predators include tits in their diet and thus pose a threat to adults, whereas none of them prey upon eggs or nestlings of cavity nesters. For the nonpredator playback treatment, we used four nocturnal and 14 diurnal bird species that occur in southern Germany, but that do not prey upon or compete for resources with blue tits (Appendix Table A1). We used a total of 281 predator recordings and 253 nonpredator recordings, obtained from the online repository Xeno Canto (www.xeno-canto.org/). To avoid pseudoreplication, each speaker in both the predator and nonpredator treatment only played half of the available calls, such that different nests were subjected to a different subset of calls.

General Field Procedures

Starting mid-March, we checked all nestboxes at least once a week to monitor the onset and progress of nest building, the date of the first egg, clutch size, the date of first hatching, brood size, the number of fledglings and the date of fledging. Fourteen days after a brood had hatched, we measured tarsus length (to the nearest 0.05 mm) and body mass (to the nearest 0.1 g) of all nestlings and took a small blood sample (ca. 50 μ l) from the brachial vein for parentage analysis. We also collected all dead nestlings and unhatched eggs in the nest. Each adult in the study population was blood sampled and equipped with a passive integrated transponder (PIT, BIOMARK HPT8 animal tag 134.2 kHz

FDXB, 8.4 mm \times 1.4 mm, 0.03 g, Biomark, Boise, ID, U.S.A.) which was inserted under the skin on the back (Schlicht & Kempenaers, 2015). Most individuals had already been tagged during previous breeding attempts or during the nonbreeding season (caught while sleeping in a nestbox, or, in 2015 and 2016, using mist nets near feeders just outside the study area). Only 28 individuals (15 males and 13 females, 12% of all individuals) had not been caught previously and these were caught at the nest when their nestlings were 8–12 days old. We assessed the parentage of all offspring by comparing the genotypes from parents and their putative offspring using a set of 11 microsatellite markers (see Schlicht, Grg, Loès, Valcu, & Kempenaers, 2012). Of 990 eggs, 906 (92%) were successfully genotyped. Of 906 genotyped offspring, 95 (10.5%) were sired by an extrapair male and 45 of the 106 broods (42%) contained at least one extrapair offspring. For a more detailed description of the study site and general field procedures, see Schlicht et al. (2012).

Nest Visit Monitoring

We used an automated monitoring system that recorded all nestbox visits from PIT-tagged individuals throughout the breeding period, as described in Schlicht et al. (2012). Briefly, all nestboxes were equipped with an RFID reader, a real-time clock, two light barriers (one on the inside and one on the outside of the entrance hole) and a data storage device. Whenever a tagged bird passed through the nest hole, its identity and the associated time and date were recorded. The order in which the light barriers were triggered allowed us to assess whether the bird entered or exited the nestbox. From these data, we extracted (1) the time of first emergence from the nestbox in the morning (for males and females that spent the night in a nestbox), (2) date and time of each male and female visit to a nestbox occupied by a different pair (extrabox visit), and we estimated (3) time spent incubating by the female and incubation feeds by the male (as described in Bambini, Schlicht, & Kempenaers, 2018) and (4) male and female provisioning rate (as the daily number of times parents entered the nestbox during the nestling period).

Statistical Analyses

All statistical analyses were performed with R (versions 3.1.2 and later; R Development Core Team, 2014). For variables that included repeated measures of the same subject, we used the lme4 package for mixed-effects models (version 1.1–13, Bates, Maechler, Bolker, & Walker, 2015). The data from the north (predator) and south (nonpredator) plots during the playback year (2016) were combined with data from the north and south plots from previous, nonplayback years. In all models, we included 'treatment' as an explanatory variable with four levels: 2016 north (predator playback), 2016 south (nonpredator playback), ≤ 2015 north (no playback), ≤ 2015 south (no playback). We used the glht function from the 'multcomp' package (version 1.4–6, Hothorn, Bretz, & Westfall, 2008) for evaluation of the following, preselected contrasts: (1) plot effect in playback year, gauged by the difference between the north (predator) and south (nonpredator) plots in the playback year ($north_{2016} - south_{2016}$); (2) plot effect in previous, nonplayback years, gauged by the difference between the north and south plots in previous, nonplayback years ($north_{\leq 2015} - south_{\leq 2015}$); (3) year effect, gauged by the difference between the playback year (2016) and previous nonplayback years, combining the data from both plots ($(north_{2016} + south_{2016}) - (north_{\leq 2015} + south_{\leq 2015})$); (4) plot*year interaction, gauged by testing whether the difference between the north (predator) and the south (nonpredator) plot was significantly larger in the playback year (2016) than in the previous, nonplayback

years ((north₂₀₁₆–south₂₀₁₆)–(north_{<2015}–south_{<2015})). The fit of all models was evaluated by visually assessing model residuals.

Reproduction and reproductive success

For the response variables laying date (days from 1 April) and clutch size, we performed linear models with treatment as the fixed effect. To test the effects of the treatment on hatching and fledging success, we first examined whether any hatchlings/fledglings were produced using a generalized linear model with a binomial error structure with success (yes/no) as the response variable and with treatment and clutch size or number of hatchlings as fixed effects. For the successful clutches (i.e. where number of hatchlings/fledglings was >0), we then examined the proportion of eggs that hatched and the proportion of hatchlings that fledged using a generalized linear model with a binomial error structure with the number of hatchlings/fledglings as the response variable and with clutch size/the number of hatchlings as the binomial denominator, and treatment as a fixed effect. Finally, we examined whether the per capita reproduction (i.e. the average number of fledglings across all nests in a plot) differed between the predator (north) and nonpredator (south) plots in the playback year (2016) and between the north and south plots in the nonplayback years. Here, we used nonparametric Mann–Whitney *U* tests, because the data distribution did not allow parametric tests (disproportionate number of zeros). We additionally tested, separately for the north and the south plots, whether per capita reproduction differed between the playback year (2016) and the nonplayback years. Replacement clutches ($N = 12$, 1.8% of all clutches between 2010 and 2016, one case in each plot in 2016) were excluded from all analyses, as were nests with fewer than four eggs ($N = 13$ cases, 2.0% of all clutches, one case in each plot in 2016) which are never successful and therefore considered unfinished.

Offspring Quality and Parental Care

Offspring size and condition

We tested effects of the treatment on offspring tarsus length (reflecting growth) using a linear mixed model with tarsus length (at 14 days of age) as the response variable, treatment and brood size as fixed effects and nest ID as a random intercept. To test the effect of the treatment on nestling condition, we used a similar model with body mass as the response variable, but with tarsus length as an additional explanatory variable to control for variation in body mass that is due to size.

Incubation

For females, we extracted for each day of the incubation period the total amount of time she spent inside the nestbox between 0700 and 2000. We restricted our data to these hours to exclude presence in the nestbox associated with sleep. For males, we extracted for each day of the incubation period the total number of visits made to the nestbox (presumably to feed their mate; see [Bambini et al., 2018](#)). We defined the incubation period as starting 14 days before hatching until the day of hatching. Thus, we only included nests where at least one egg hatched. We performed linear mixed models with time (h) spent incubating or number of visits as the response variable, treatment, incubation day and incubation day squared (to allow for a nonlinear effect, see [Bambini et al., 2018](#)) as fixed effects and individual ID as a random intercept.

Nestling provisioning

For both females and males we extracted for each day of the nestling period the total number of visits made to the nestbox. We defined the nestling period as starting on the day after hatching until nestlings were 18 days old. We only included nests that were

successful, that is, where at least one nestling fledged. We performed linear mixed models (separately for each sex) with daily number of visits as the response variable, treatment, brood size, nestling age and nestling age squared (to allow for a nonlinear effect) as fixed effects and individual ID as a random intercept. Some parents (12%) were trapped during the nestling provisioning period and this may have affected their nestbox visit rate or their response to the treatment. However, running the same analyses on the subset of data that excluded these 24 individuals gave qualitatively similar results (details not shown).

Extrapair Paternity and Behaviour

Extrapair paternity

For females, we examined the likelihood that their nest contained at least one extrapair offspring (EPO). For males, we examined the likelihood that they had sired at least one EPO. We used generalized linear models (for each sex separately) with a binomial error structure with EPO (yes/no) as the response variable and with treatment and brood size as fixed effects. In the model examining male EPP, we additionally included male age (yearling or adult) as a fixed effect, because male age strongly influences the likelihood of gaining EPP ([Poesel et al., 2006](#); [Schlicht et al., 2015a, b](#)).

Extrabox visits

For each pair, we determined (1) whether the female visited a nestbox occupied by another pair during her fertile period and (2) whether the pairs' nestbox was visited by a male other than the social male during the fertile period of the resident female. We defined the fertile period as the period from 5 days to 1 day before the start of egg laying (days -5 to -1; see [Schlicht et al., 2014](#) for further explanation), but using other definitions (i.e. days -2 and -1, [Schlicht et al., 2014](#)) gave qualitatively the same results (not shown). We performed generalized linear models (separately for visits made and visits received) with a binomial error structure. Whether or not an extrabox visit took place (yes/no) was the response variable, and we included treatment as a fixed effect. Extrabox visits only occurred between individuals of the same plot, that is, females never visited a box outside their plot and nests were never visited by a male from outside the plot.

Timing of emergence

For females, we extracted for each day of her fertile period (defined as days -5 to -1, see above) the timing of emergence from the nestbox. For males, we extracted for each day of the fertile period of his mate the timing of emergence from his sleeping box. We used linear mixed models (separately for each sex) with emergence time (converted to minutes relative to sunrise, see [Steinmeyer, Schielzeth, Mueller, & Kempenaers, 2010](#)) as the response variable, treatment as a fixed effect and individual ID as a random intercept. In contrast to females, males never slept in the breeding box, but they occasionally spent the night in unoccupied boxes. The sample size for males is therefore substantially lower than that for females. Using a more restricted definition of the fertile period (i.e. days -2 and -1, see above) gave qualitatively the same results (not shown). For males, we additionally performed an analysis that included all emergence times during the period when any female in the population (not just his mate) was within 5 days from egg laying, but the results were again qualitatively the same (not shown).

Ethical Note

Previous studies did not find any adverse effects of subcutaneous transponder tags on the fitness of adult or nestling passerines

(Nicolaus, Bouwman, & Dingemanse, 2008; Schroeder, Cleasby, Nakagawa, Ockendon, & Burke, 2011). In our study population, too, no effects of the transponder implantation on the long- or short-term condition of adults and young were found (Steinmeyer et al., 2010) and adults do not appear exceptionally stressed during insertion of the transponder in comparison to routine procedures (Schlicht & Kempnaers, 2018). All predator species that were played back occur in southern Germany and can thus naturally be encountered at or near our study site. We did not notice any obvious responses to the predator playbacks. The study complies with the ASAB/ABS guidelines for the treatment of animals in research. Permits for data collection and carrying out the playback

experiment were issued by the Bavarian government and the Bavarian regional office for forestry (LWF).

RESULTS

Reproductive Success

There was no significant plot*year interaction with respect to laying date, clutch size, the likelihood of producing at least one hatchling, the proportion of eggs that hatched, the likelihood of producing at least one fledgling and the proportion of hatchlings that fledged (Fig. 1a–f, Table 1). In the playback year (2016) the

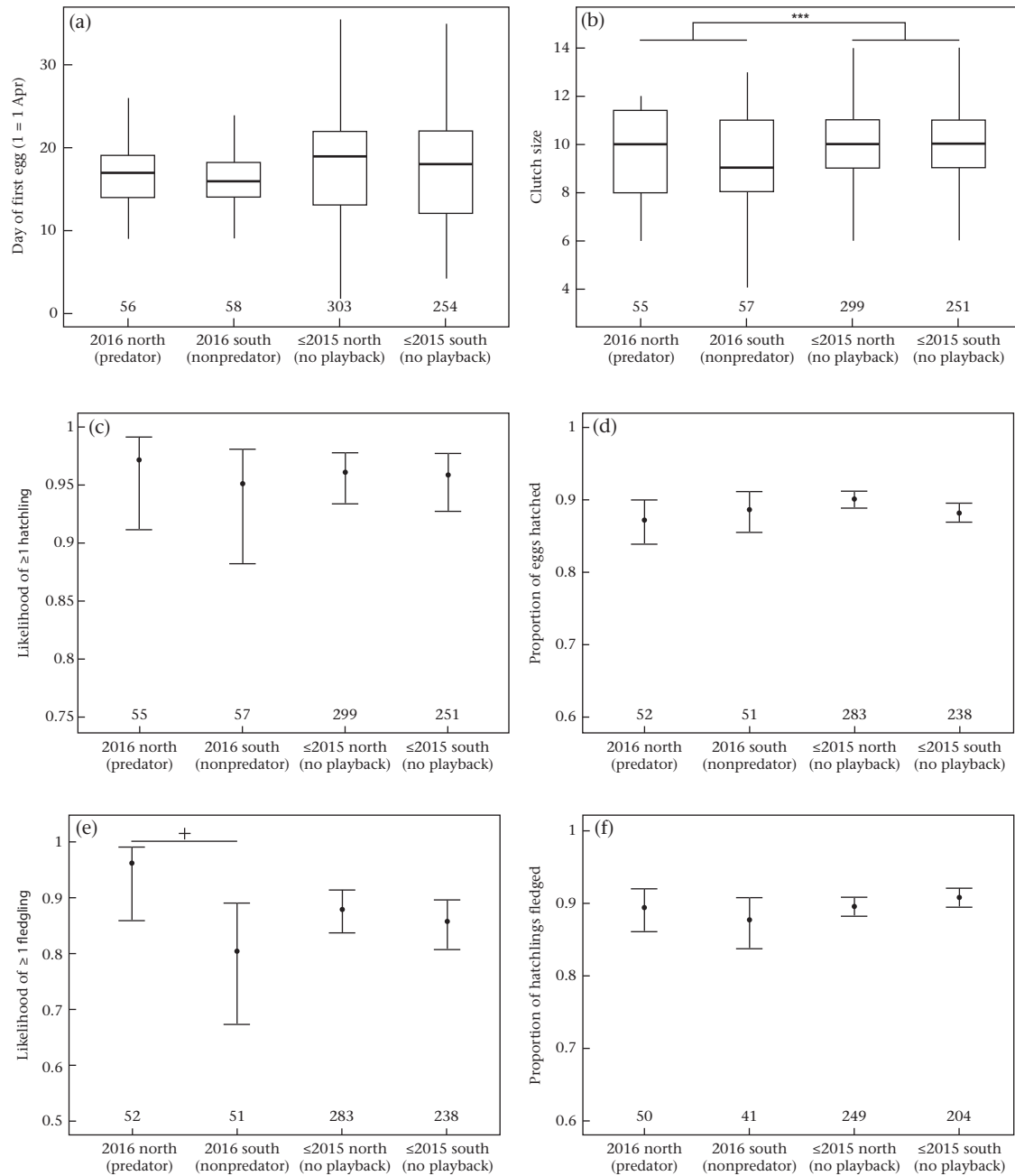


Figure 1. Comparison of measures of reproductive behaviour and success between blue tits breeding in an area where predator calls were broadcast (north) and those from a control area (playback of calls of nonpredators, south), in the experimental year (2016) and in the nonexperimental years (2010–2015). For (a) laying date and (b) clutch size, box plots are shown with the median, first and third quartiles and range (within 1.5 × the interquartile range) of the raw data. For (c) the likelihood of producing at least one hatchling, (d) the proportion of eggs that hatched (given that at least one hatched), (e) the likelihood of producing at least one fledgling and (f) the proportion of hatchlings that fledged (given that at least one hatchling fledged), model estimates are shown (points) with their 95% confidence intervals (see Methods for model details). Sample sizes (number of nests) are given below the error bars. +P < 0.10; ***P < 0.001.

Table 1
Effect of the experiment on measures of reproductive success

		Estimate	SE	t/z	P
Laying date	(Intercept)	17.71	0.96		
	Plot effect 2016	-1.18	1.37	-0.86	0.79
	Plot effect ≤ 2015	1.03	0.62	1.66	0.28
	Year effect	0.70	0.75	0.93	0.74
	Plot*year interaction	2.21	1.50	1.47	0.39
Clutch size	(Intercept)	9.27	0.25		
	Plot effect 2016	0.08	0.35	0.24	0.99
	Plot effect ≤ 2015	-0.16	0.16	-1.02	0.69
	Year effect	0.85	0.19	4.37	<0.001
	Plot*year interaction	-0.25	0.39	-0.64	0.90
Likelihood of producing ≥ 1 hatchling	(Intercept)	3.07	0.53		
	Clutch size	0.49	0.07	6.61	<0.001
	Plot effect 2016	0.43	0.79	0.54	0.93
	Plot effect ≤ 2015	-0.17	0.40	-0.42	0.97
	Year effect	-0.07	0.45	-0.16	1.00
Proportion eggs hatched	(Intercept)	2.05	0.14		
	Plot effect 2016	-0.12	0.20	-0.59	0.92
	Plot effect ≤ 2015	0.14	0.09	1.55	0.34
	Year effect	0.13	0.11	1.19	0.57
	Plot*year interaction	0.25	0.22	1.18	0.58
Likelihood of producing ≥ 1 fledgling	(Intercept)	1.41	0.35		
	Hatched	-0.02	0.06	-0.27	0.79
	Plot effect 2016	1.78	0.80	2.22	0.079
	Plot effect ≤ 2015	0.25	0.27	0.93	0.71
	Year effect	-0.35	0.43	-0.82	0.78
Proportion chicks fledged	(Intercept)	1.97	0.16		
	Plot effect 2016	0.15	0.23	0.64	0.90
	Plot effect ≤ 2015	-0.16	0.11	-1.48	0.38
	Year effect	0.19	0.13	1.52	0.36
	Plot*year interaction	-0.30	0.25	-1.21	0.56

Model summaries examining the differences in reproductive behaviour and success between the predator playback (north) and nonpredator playback (south) plot in the playback year (2016) and in the nonplayback years (2010–2015). Post hoc evaluation of the following, preselected contrasts was performed: (1) plot effect in playback year ($north_{2016} - south_{2016}$), (2) plot effect in previous, nonplayback years ($north_{\leq 2015} - south_{\leq 2015}$), (3) year effects ($(north_{2016} + south_{2016}) - (north_{\leq 2015} + south_{\leq 2015})$) and (4) plot*year interaction ($(north_{2016} - south_{2016}) - (north_{\leq 2015} - south_{\leq 2015})$). Estimates for intercept and main effects (if applicable) are derived from the main model and estimates for contrasts are derived from post hoc comparisons (see Methods for details). *t* values are given for laying date and clutch size, *z* values for likelihood of producing ≥ 1 hatchling, proportion of eggs hatched, likelihood of producing ≥ 1 fledgling, proportion of chicks fledged.

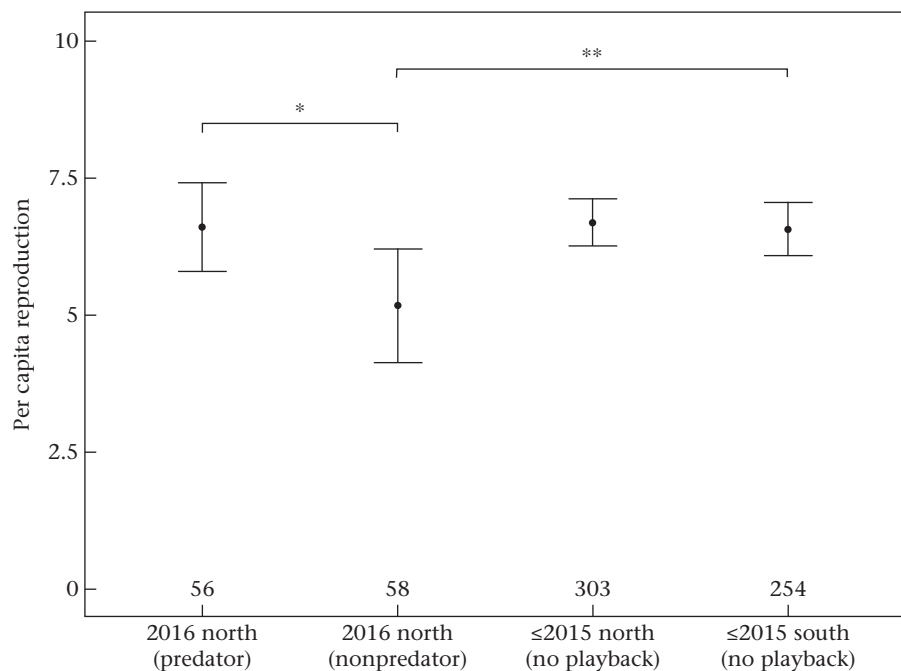


Figure 2. Comparison of per capita reproduction (i.e. the average number of fledglings across all nests in a plot) between the predator plot (north) and nonpredator plot (south), in the playback year (2016) and in the nonplayback years (2010–2015). Error bars represent means (points) and 95% confidence intervals. Sample sizes (number of nests) are given below the error bars. * $P < 0.05$; ** $P < 0.01$.

likelihood of producing at least one fledgling was somewhat higher in the predator plot (north: 96% of the nests; 50/52) than in the nonpredator plot (south: 80% of the nests; 41/51), although this difference was not statistically significant (Fig. 1e, Table 1).

In the playback year, per capita reproduction (i.e. the average number of fledglings across all nests in a plot) was significantly higher for nests in the predator plot compared to nests in the nonpredator plot (Mann–Whitney *U* test: $W = 1212$, $P = 0.037$; Fig. 2). This was not the case in the nonplayback years (Mann–Whitney *U* test: $W = 38\,015$, $P = 0.79$; Fig. 2). In the nonpredator plot (south), per capita reproduction was significantly lower in the experimental year than in nonexperimental years (Mann–Whitney

U test: $W = 8971.5$, $P = 0.002$; Fig. 2), but this was not the case in the predator plot (Mann–Whitney *U* test: $W = 8920$, $P = 0.31$; Fig. 2).

Offspring Quality and Parental Care

There was no significant plot*year interaction in offspring quality, as measured by nestling tarsus length and mass (Fig. 3a and b, Table 2). There was also no significant plot*year interaction with respect to female incubation, incubation feeding of females by males or the nest visit rate by females during the nestling period (Fig. 3c–e, Table 2). In the playback year (2016) males in the

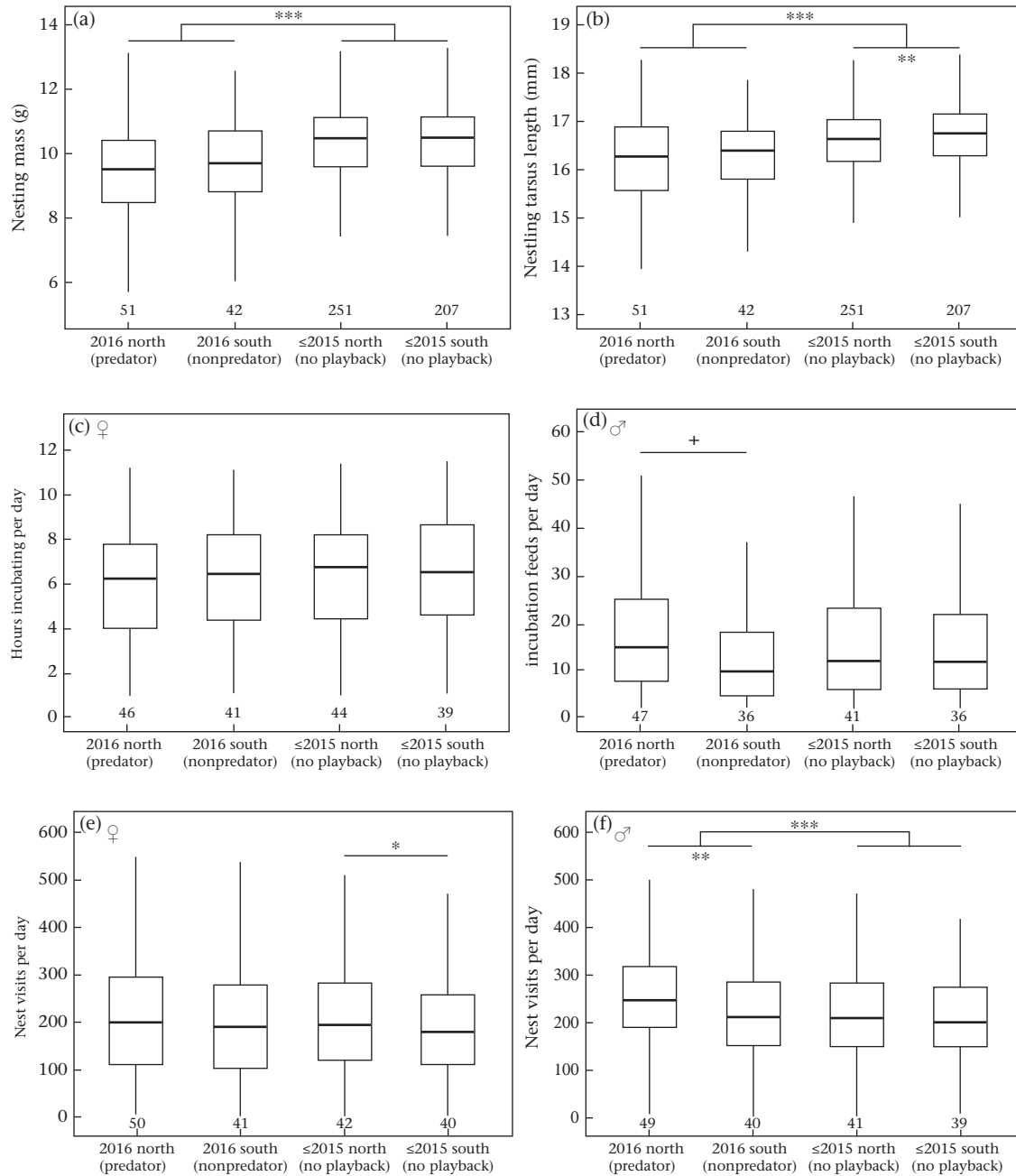


Figure 3. Comparison of nestling quality and measures of parental care between blue tits breeding in an area where predator calls were broadcast and those from a control area where calls of nonpredators were broadcast (south), in the experimental year (2016) and in the nonexperimental years (2010–2015 or 2015 only). (a) Nestling mass, (b) nestling tarsus length, (c) incubation by female, (d) male incubation feeds, (e) nest visits by female and (f) nest visits by male. Box plots show the median, first and third quartiles and range (within $1.5 \times$ the interquartile range) of the raw data. Sample sizes (number of nests) are given below the error bars. + $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2
Effect of the experiment on nestling condition and parental care

		Estimate	SE	<i>t</i>	<i>P</i>
Nestling tarsus	(Intercept)	16.26	0.09		
	Brood size	-0.06	0.01	-5.59	<0.001
	Plot effect 2016	-0.12	0.12	-1.01	0.69
	Plot effect ≤2015	-0.16	0.05	-3.12	0.007
	Year effect	0.38	0.06	5.90	<0.001
Nestling mass	Plot*year interaction	-0.04	0.13	-0.34	0.98
	(Intercept)	-7.80	0.27		
	Brood size	-0.15	0.01	-11.22	<0.001
	Tarsus length	1.06	0.02	70.85	<0.001
	Plot effect 2016	-0.05	0.14	-0.33	0.98
Female incubation hours/day	Plot effect ≤2015	0.06	0.06	1.03	0.68
	Year effect	0.45	0.08	5.88	<0.001
	Plot*year interaction	0.11	0.15	0.72	0.86
	(Intercept)	7.02	0.17		
	Incubation day	0.23	0.01	41.19	<0.001
Male incubation feeds/day	(Incubation day) ²	-0.03	0.00	-29.70	<0.001
	Plot effect 2016	-0.12	0.24	-0.53	0.94
	Plot effect ≤2015	0.06	0.24	0.27	0.99
	Year effect	0.20	0.17	1.17	0.59
	Plot*year interaction	0.19	0.34	0.56	0.93
Female nest visits/day	(Intercept)	14.76	1.66		
	Incubation day	0.07	0.09	0.82	0.41
	(Incubation day) ²	-0.07	0.02	-2.82	0.005
	Plot effect 2016	4.97	2.14	2.33	0.068
	Plot effect ≤2015	0.87	2.21	0.39	0.97
Male nest visits/day	Year effect	-0.97	1.54	-0.63	0.90
	Plot*year interaction	-4.11	3.08	-1.33	0.48
	(Intercept)	242.07	7.52		
	Chick age	15.37	1.53	10.03	<0.001
	(Chick age) ²	10.81	0.49	22.28	<0.001
Female nest visits/day	Brood size	-1.18	0.05	-25.37	<0.001
	Plot effect 2016	14.29	11.53	1.24	0.54
	Plot effect ≤2015	31.39	11.96	2.62	0.031
	Year effect	-17.33	8.41	-2.06	0.13
	Plot*year interaction	17.09	16.61	1.03	0.68
Male nest visits/day	(Intercept)	242.97	8.38		
	Chick age	18.21	1.86	9.80	<0.001
	(Chick age) ²	6.28	0.24	25.96	<0.001
	Brood size	-0.82	0.05	-16.07	<0.001
	Plot effect 2016	37.54	11.08	3.39	0.003
Male nest visits/day	Plot effect ≤2015	23.75	11.65	2.04	0.13
	Year effect	-34.56	8.11	-4.26	<0.001
	Plot*year interaction	-13.78	16.07	-0.86	0.79

Model summaries examining the differences in nestling tarsus length and body mass (controlled for tarsus length) and measures of parental care in the predator playback (north) and the nonpredator playback (south) plot in the playback year (2016) and in the nonplayback years (2010–2015). Post hoc evaluation of the following preselected contrasts was performed: (1) plot effect in playback year ($\text{north}_{2016} - \text{south}_{2016}$), (2) plot effect in previous, nonplayback years ($\text{north}_{<2015} - \text{south}_{<2015}$), (3) year effects ($(\text{north}_{2016} + \text{south}_{2016}) - (\text{north}_{<2015} + \text{south}_{<2015})$) and (4) plot*year interaction ($(\text{north}_{2016} - \text{south}_{2016}) - (\text{north}_{<2015} - \text{south}_{<2015})$). Estimates for intercept and main effects are derived from the main model and estimates for contrasts are derived from post hoc comparison (see Methods for details).

predator plot made significantly more nest visits per day during the nestling period than those in the nonpredator plot. However, there was a similar difference between the north and the south plot in the previous (nonplayback) year, and there was indeed no significant plot*year interaction effect (Fig. 3f, Table 2).

Extrapair Paternity and Behaviour

There was no significant plot*year interaction on the likelihood of having at least one extrapair offspring (Fig. 4a and b, Table 3). There was also no significant plot*year interaction for the probability that a female visited another nestbox, for the probability that a nestbox was visited by an extrapair male, or for the time that either females or males emerged from the nestbox in the morning (Fig. 4c–f, Table 3).

Predator Presence

In the year of the playbacks, we observed an avian predator on 16 occasions in the predator plot (0.16/h) and

on 15 occasions in the nonpredator plot (0.15/h; Appendix Table A2). The rate of predator sightings in the predator and the nonpredator plots was thus almost identical, suggesting that the presence of avian predators did not differ between the plots.

Interannual Differences

Several measures differed significantly between the playback year (2016) and previous, nonplayback years. Clutches were smaller in the playback year than in the previous, nonplayback years (Fig. 1b, Table 1). Nestling tarsus length and mass (controlled for tarsus length) were also lower in the playback year than in nonplayback years (Fig. 3a and b, Table 2) and males visited their nest less frequently during the nestling provisioning period in the playback year (Fig. 3f, Table 2). Finally, the emergence time of females was later in the playback year than in the nonplayback year (Fig. 4e and f, Table 3).

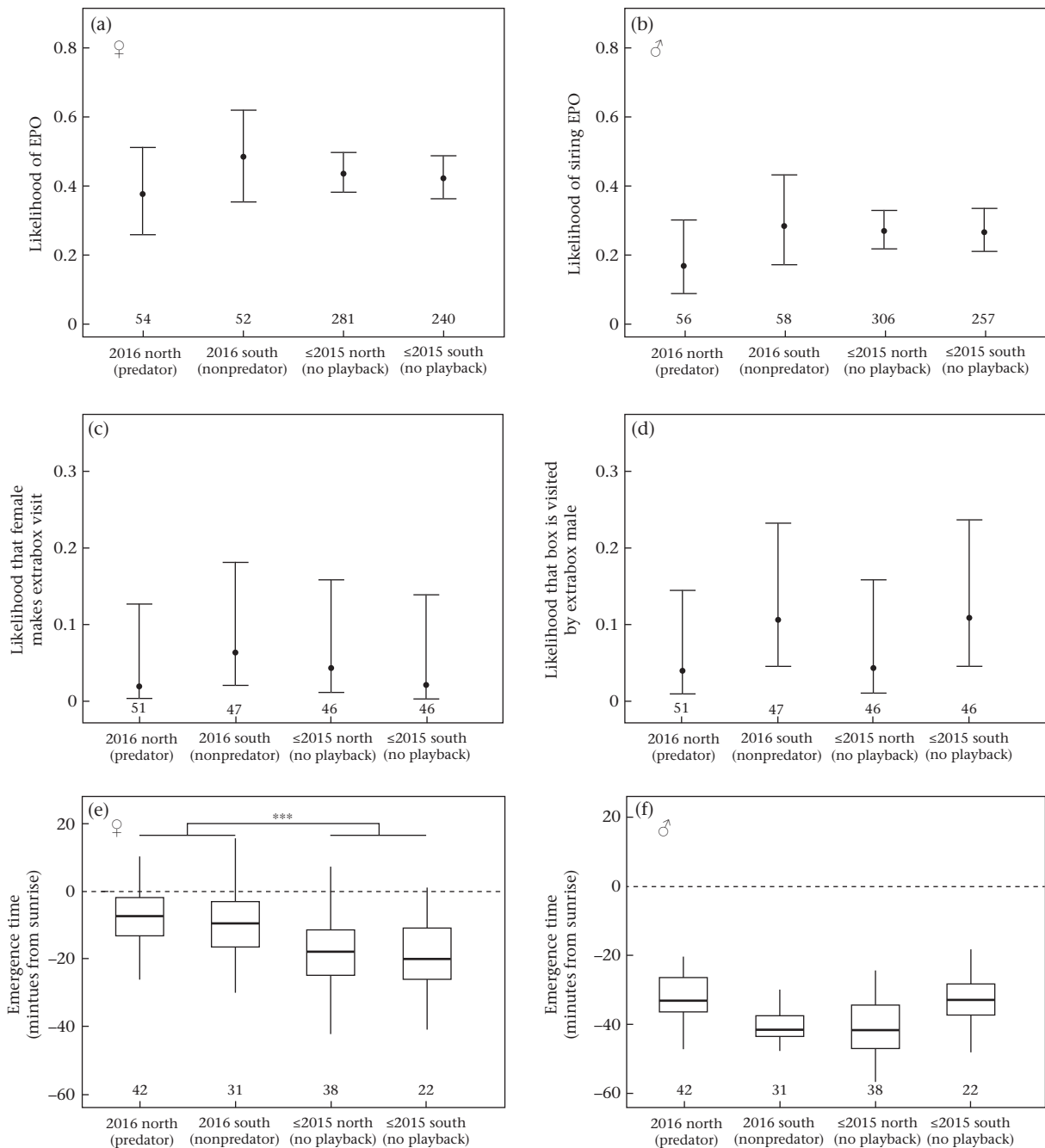


Figure 4. Comparison of extrapair paternity and related behaviours between blue tits breeding in an area where predator calls were broadcast (north) and those from a control area where calls of nonpredators were broadcast (south), in the experimental year (2016) and in the nonexperimental years (2010–2015 or 2015 only). For (a) female likelihood of having at least one extrapair offspring (EPO), (b) male likelihood of siring at least one EPO, (c) daily likelihood of an extrabox visit by females and (d) daily likelihood that a box is visited by an extrabox male model estimates are shown (points) with their 95% confidence intervals (see Methods for model details). For (e) female and (f) male emergence time from the nestbox box plots show the median, first and third quartiles and range (within $1.5 \times$ the interquartile range) of the raw data. Sample sizes (number of individuals) are given below the error bars. *** $P < 0.001$.

DISCUSSION

We found no significant effect of a predator playback treatment on the timing of reproduction, clutch size, hatching success and most reproductive behaviours of the blue tits in our population. Male nest visit rate in the playback year (2016) differed significantly between the predator (north) and the nonpredator (south) plot, but this difference between plots was similar in the nonplayback year and there was indeed no significant plot*year interaction effect.

These findings are in contrast to most previous studies that manipulated perceived predation risk and reported changes in reproductive investment (Basso & Richner, 2015; Fontaine & Martin, 2006; Ghalambor et al., 2013; Ghalambor & Martin, 2002; Julliard et al., 1997; LaManna & Martin, 2016; Zanette et al., 2011). We also found no significant effect of the predator playback treatment on behaviours that may indicate extrapair activity in blue tits, namely morning emergence time and extrabox visits, or on the occurrence of EPP. This is consistent with a recent study in

Table 3
Effect of the experiment on extrapair paternity and related behaviours

		Estimate	SE	t/z	P
Female EPP	(Intercept)	-0.10	0.28		
	Brood size	0.04	0.04	1.05	0.30
	Plot effect 2016	-0.41	0.40	-1.03	0.68
	Plot effect ≤ 2015	0.11	0.18	0.60	0.91
	Year effect	0.01	0.22	0.05	1.00
	Plot*year interaction	0.52	0.44	1.18	0.57
Male EPP	(Intercept)	-3.02	0.48		
	Age	1.35	0.21	6.36	<0.001
	Plot effect 2016	-0.66	0.50	-1.31	0.49
	Plot effect ≤ 2015	0.01	0.21	0.06	1.00
	Year effect	0.26	0.27	0.94	0.74
	Plot*year interaction	0.67	0.55	1.23	0.54
Likelihood extrabox visit female	(Intercept)	-2.69	0.60		
	Plot effect 2016	-1.23	1.17	-1.05	0.66
	Plot effect ≤ 2015	0.72	1.24	0.58	0.92
	Year effect	-0.15	0.85	-0.18	1.00
	Plot*year interaction	1.94	1.71	1.14	0.60
	(Intercept)	-2.13	0.47		
Likelihood extrabox visit male	Plot effect 2016	-1.07	0.86	-1.24	0.53
	Plot effect ≤ 2015	-0.99	0.86	-1.14	0.59
	Year effect	0.07	0.61	0.11	1.00
	Plot*year interaction	0.08	1.22	0.07	1.00
	(Intercept)	-7.85	1.89		
	Emergence time female	Plot effect 2016	1.81	2.43	0.75
Plot effect ≤ 2015		-0.98	2.73	-0.36	0.98
Year effect		-8.27	1.83	-4.53	<0.001
Plot*year interaction		-2.80	3.66	-0.77	0.84
(Intercept)		-37.69	12.24		
Emergence time male		Plot effect 2016	16.09	13.99	1.15
	Plot effect ≤ 2015	-8.03	11.08	-0.72	0.85
	Year effect	2.90	8.92	0.33	0.98
	Plot*year interaction	-24.12	17.84	-1.35	0.45

Model summaries examining the differences in extrapair paternity (EPP) and related behaviours between the predator playback (north) and the nonpredator playback (south) plot in the playback year (2016) and in the nonplayback years (2010–2015). Post hoc evaluation of the following preselected contrasts was performed: (1) plot effect in playback year ($north_{2016} - south_{2016}$), (2) plot effect in previous, nonplayback years ($north_{\leq 2015} - south_{\leq 2015}$), (3) year effects ($(north_{2016} + south_{2016}) - (north_{\leq 2015} + south_{\leq 2015})$) and (4) plot*year interaction ($(north_{2016} - south_{2016}) - (north_{\leq 2015} - south_{\leq 2015})$). Estimates for intercept and main effects (if applicable) are derived from the main model and estimates for contrasts are derived from post hoc comparison (see Methods for details). *t* values are given for female and male EPP, female and male likelihood of extrabox visits; *z* values are given for female and male emergence time.

which sparrowhawk calls were played throughout the breeding period to great tits, *Parus major* (Abbey-Lee et al., 2018). This study also reported no effect of predator playbacks on rates of EPP, even though the treatment was clearly perceived as an increase in predation risk as indicated by changes in singing and exploration behaviour (Abbey-Lee et al., 2016; Abbey-Lee Mathot et al., 2016). The sole significant difference we found between the predator and nonpredator playback plots in the playback year, which is not attributable to a plot effect, was that more fledglings were produced on the predator playback plot.

This latter result was associated with fewer instances of complete brood mortality in the predator playback plot (2/52 broods) compared to the nonpredator playback plot (10/51 broods). We have previously shown that complete brood mortality in our population results almost exclusively from the sudden disappearance of one of the parents (probably due to predation by sparrowhawks), followed by abandonment or an inability of the remaining parent to raise the brood alone (Santema & Kempenaers, 2018). Indeed, in all seven (of 12) failed nests in the current study for which data of parental visits were available, one of the parents had suddenly and permanently disappeared from the population during nestling provisioning. The greater number of fledglings and fewer complete brood failures on the predator playback plot could thus be simply due to chance, for example resulting from a single sparrowhawk being active in the nonpredator playback plot, but not in the predator playback plot. It is also conceivable that the predator calls in the predator playback plot deterred actual predators, or that the playback of calls of potential prey species attracted predators to the

nonpredator plot. However, behavioural observations indicate that the presence of avian predators did not differ between the two plots (see Results).

A further potential explanation for why we found almost no behavioural differences between the predator and the nonpredator playback plots is that the fast life history of blue tits may make them less sensitive to changes in predation risk (Ghalambor & Martin, 2001; LaManna & Martin, 2016). Life history theory predicts that parents with a low probability of breeding again should tolerate greater risk, even if this comes at a cost to the current breeding attempt (Clark, 1994; Ghalambor & Martin, 2001). Blue tits breed only once per year in our population and only about half of the adults survive each winter. Many blue tits therefore only reproduce once in their lifetime. As a consequence, it seems unlikely that individuals benefit from adopting a risk-averse strategy in favour of future breeding attempts. The importance of future reproductive opportunities was recently demonstrated by a study examining the effects of predation risk on 10 different songbird species simultaneously (LaManna & Martin, 2016). This study showed that under increased predation risk the reduction in investment in the current breeding attempt was positively related to the birds' residual reproductive value, that is, to the probability that they will breed again.

Another explanation for the general absence of differences between the predator playback and the nonpredator playback plots is that the experimental playbacks did not change the perceived predation risk of the blue tits, for example due to habituation to the calls. However, habituation did not appear to play a role in previous

playback studies whose protocol we closely followed (Hua et al., 2014; LaManna & Martin, 2016; Zanette et al., 2011). Alternatively, blue tits (and other cavity-nesting species) might be less sensitive to changes in predation risk, because the cavity (nestbox) provides better protection from predators (Fontaine & Martin, 2006; Julliard et al., 1997; Martin & Briskie, 2009). Lastly, our presence in the study area and the fact that we catch all individuals and regularly check their nestboxes may already have led to an increase in their perceived risk of predation, such that broadcasting predator calls may have had little additional effect.

Finally, the general absence of an effect of the playbacks on behaviour may be related to the environmental circumstances in the year the experiment was carried out. Indeed, the breeding season of 2016 appeared to be an unusually poor one, as indicated by significantly smaller clutches (Table 1) and significantly lower nestling mass and tarsus length compared to previous, nonplayback years (Table 2). This may be related to unusually late snowfall during the egg-laying phase (25–27 April 2016), which caused many birds to temporarily halt laying and may have had subsequent effects on food availability during the nestling period. Male nest visit rates during the nestling period were significantly higher in the playback year than in the previous nonplayback year, suggesting that males might have attempted to compensate for a lower quality of food by provisioning at a higher rate. Numerous studies on diverse taxa have previously demonstrated that food and predators interact in affecting demography, whereby the effects of perceived predation risk are reduced when food is in short supply (Karels, Byrom, Boonstra, & Krebs, 2000; Krebs et al., 1995; Preisser, Bolnick, & Grabowski, 2009; Zanette, Smith, van Oort, & Clinchy, 2003). Thus, it would be useful to perform predation risk manipulations in multiple years and assess whether (and if so, how) the effect of perceived risk is modulated by environmental conditions.

Conclusions

In contrast to many previous studies demonstrating that perceived predation risk affects various aspects of reproductive investment in songbirds, we found no evidence that playback of predator calls affected reproductive behaviour and success of blue tits. We also found no evidence that playback of predator calls affected indices of extrapair behaviour and the level of EPP, which is consistent with the only previous study that examined the effect of perceived predation risk on EPP in the closely related great tit (Abbey-Lee et al., 2018). We propose two general, mutually exclusive explanations for the general absence of any effects. First, the perceived predation risk did not differ between the predator and nonpredator playback plots, for example because our presence already caused blue tits to perceive their environment as high risk. Second, the perceived predation risk was higher in the predator playback area, but blue tits (and great tits) did not respond to this, for example because they do not benefit from adopting a risk-averse strategy at a cost to their current reproductive attempt. Because the interpretation of these results remains difficult, or at least speculative, further studies either with different experimental approaches (e.g. using visual stimuli and/or targeted manipulation during the period when extrapair copulations take place) or on species with a different life history are needed to assess whether perceived predation risk has the potential to influence the occurrence of EPP.

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Appendix

Table A1
Predator and nonpredator species that were used for the playback treatments

Predator species (experiment)	Nonpredator species (control)
Barn owl, <i>Tyto alba</i>	Black-headed gull, <i>Chroicocephalus ridibundus</i>
Black kite, <i>Milvus migrans</i>	Black-tailed godwit, <i>Limosa limosa</i>
Common buzzard, <i>Buteo buteo</i>	Collared dove, <i>Streptopelia decaocto</i>
Goshawk, <i>Accipiter gentilis</i>	Common moorhen, <i>Gallinula chloropus</i>
Great grey shrike, <i>Lanius excubitor</i>	Common redshank, <i>Tringa totanus</i>
Hobby, <i>Falco subbuteo</i>	Common tern, <i>Sterna hirundo</i>
Kestrel, <i>Falco tinnunculus</i>	Common sandpiper, <i>Actitis hypoleucos</i>
Little owl, <i>Athene noctua</i>	Corn crane, <i>Crex crex</i>
Long-eared owl, <i>Asio otus</i>	Eurasian coot, <i>Fulica atra</i>
Marsh harrier, <i>Circus aeruginosus</i>	Eurasian curlew, <i>Numenius arquata</i>
Merlin, <i>Falco columbarius</i>	Golden plover, <i>Pluvialis apricaria</i>
Peregrine, <i>Falco peregrinus</i>	Little Ringed plover, <i>Charadrius dubius</i>
Red kite, <i>Milvus milvus</i>	Night heron, <i>Nycticorax nycticorax</i>
Red-backed shrike, <i>Lanius collurio</i>	Nightjar, <i>Caprimulgus europaeus</i>
Scops owl, <i>Otus scops</i>	Nutcracker, <i>Nucifraga caryocatactes</i>
Sparrowhawk, <i>Accipiter nisus</i>	Spotted crane, <i>Porzana porzana</i>
Tawny owl, <i>Strix aluco</i>	Wood pigeon, <i>Columba palumbus</i>
Tengmalm's owl, <i>Aegolius funereus</i>	Woodcock, <i>Scolopax rusticola</i>

Table A2
Observations of avian predators during the playback year (2016)

Date	Plot	Species	Number	Behaviour
30 Mar	Predator	Buzzard	2	Flying over
30 Mar	Predator	Buzzard	1	Flying over
30 Mar	Predator	Buzzard	2	Flying over
1 Apr	Predator	Buzzard	1	Flying over
6 Apr	Predator	Buzzard	1	Flying over
21 Apr	Predator	Buzzard	1	Flying/perching
21 Apr	Predator	Sparrowhawk	1	Flying over
22 Apr	Predator	Buzzard	1	Flying over
23 Apr	Predator	Black kite	1	Flying over
5 May	Predator	Red kite	2	Flying over
6 May	Predator	Buzzard	1	Flying over
10 May	Predator	Buzzard	1	Flying over
17 May	Predator	Buzzard	1	Flying over
18 May	Predator	Buzzard	1	Flying over
26 May	Predator	Buzzard	1	Flying over
30 May	Predator	Buzzard	1	Flying through forest
6 Apr	Nonpredator	Buzzard	1	Flying over
8 Apr	Nonpredator	Buzzard	1	Flying over
10 Apr	Nonpredator	Buzzard	1	Flying/perching
16 Apr	Nonpredator	Buzzard	1	Flying over
22 Apr	Nonpredator	Buzzard	1	Flying over
25 Apr	Nonpredator	Kite sp.	1	Flying over
28 Apr	Nonpredator	Buzzard	1	Flying over
29 Apr	Nonpredator	Buzzard	1	Flying over
30 Apr	Nonpredator	Buzzard	1	Perching
30 Apr	Nonpredator	Buzzard	1	Flying through forest
2 May	Nonpredator	Buzzard	1	Flying over
13 May	Nonpredator	Tawny owl	1	Perching
25 May	Nonpredator	Tawny owl	1	Perching
30 May	Nonpredator	Buzzard	1	Flying over
2 Jun	Nonpredator	Buzzard	2	Flying over

Overview of avian predator sightings in the plot where predator calls were broadcast and the plot where nonpredator calls were broadcast. Sightings of avian predator species were recorded in each plot for 2 h daily (every second day after clutch completion) by walking through each plot in a systematic way that covered the entire plot (total observation time: 100 h per plot).

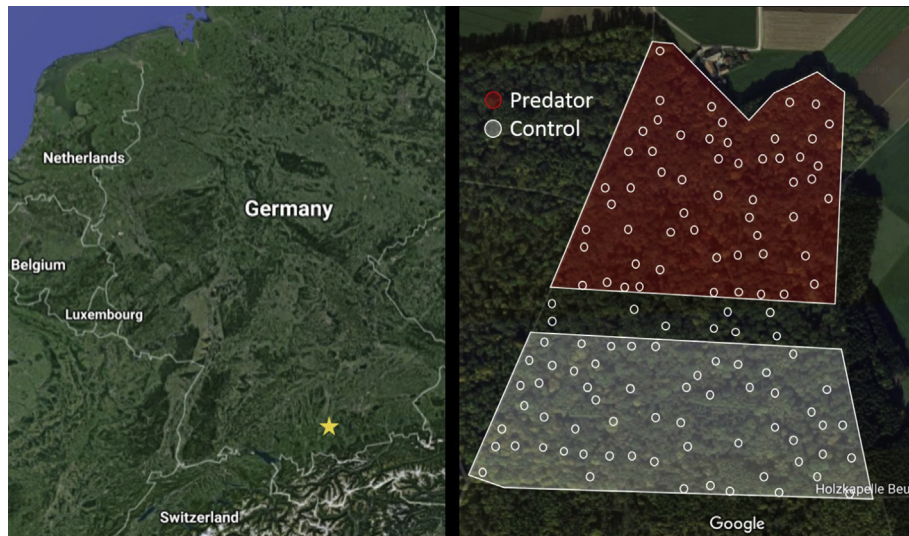


Figure A1. (a) Map indicating the location of the study area (star) in southern Germany and (b) the location of the predator and nonpredator plots and the buffer zone within the study area. Circles indicate the location of nests.