



# Parental sex allocation and sex-specific survival drive offspring sex ratio bias in little owls

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## Abstract

Although biased offspring sex ratios are common in species with sexual size dimorphism, the proximate causes are often unresolved. This is because two general mechanisms operating in different ways and in various periods of reproduction can lead to the bias: sex-biased survival or parental sex-allocation. We examined nestling sex ratio patterns between hatching and fledging, sexual size dimorphism, and factors affecting nestling survival using growth and survival data of 846 individual little owl *Athene noctua* nestlings with known sex from 307 broods from Germany, the Netherlands and Denmark. Nestling sex ratio was female-biased, mainly due to a significant female bias in the first-hatched chicks. Females showed a higher body weight than male nestlings at ringing and body weight of nestlings decreased with hatching sequence. Nestling survival was higher in females ( $\Phi = 0.91$ ) than in males ( $\Phi = 0.85$ ), and survival rates were positively related to body mass and negatively to brood size. Although the observed lower survival of males can cause an overall female-biased sex ratio, the sex dimorphism and survival patterns found here are unlikely to explain the conspicuous sex ratio pattern with a female bias in the first-hatched nestlings and the increase in female bias across the season. Thus, these results point towards interacting mechanisms of parental sex allocation strategies and sex-specific survival. As the female bias was allocated to the first rank that is most likely to survive, the female bias will increase

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under suboptimal breeding conditions. We therefore suggest that under suboptimal ecological conditions, higher investment into females is adaptive in little owls.

### Significance statement

Biased sex ratios can have severe effects on the social behaviour and population dynamics of endangered species. However, the existence of subtle sex ratio bias is often unknown and its proximate mechanisms and ultimate consequences often remain unclear. Small sample sizes make the detection of subtle effects unlikely and often fail to disentangle diverging mechanisms such as sex-biased survival and parental sex allocation. We used a large dataset of 846 little owl nestlings from 307 broods from three countries to investigate offspring sex ratio patterns, sexual size dimorphism and nestling survival simultaneously. Our findings suggest interacting mechanisms of parental sex allocation strategies and sex-specific survival to drive biased offspring sex ratios in little owls. The context dependence of the sex ratio bias indicates that offspring sex ratio bias in little owls is both, a consequence of—and an adaptation to—suboptimal breeding conditions.

**Keywords** Birds · Hatching order · Nestling survival · Parental investment · Sex-specific mortality · Sexual size dimorphism

## Introduction

Equal costs of rearing sons and daughters make balanced offspring sex ratios adaptive, as selection favours equal investment in both sexes (Darwin 1871; Fisher 1930). Yet, significant deviations from balanced offspring sex ratios are common and can be associated with intrinsic, social, environmental and reproductive factors (Hasselquist and Kempenaers 2002; Komdeur and Pen 2002; West 2002). Thereby, an unbalanced offspring sex ratio can result from two basic mechanisms: either from sex-biased offspring survival (Hasselquist and Kempenaers 2002; Komdeur and Pen 2002) or from parental sex allocation strategies that evolve due to unequal fitness of the sexes (Leimar 1996). However, the proximate mechanisms underlying biased offspring sex ratios and its ultimate consequences often remain unclear.

In altricial birds, biased offspring sex ratios can develop during three periods of reproduction. First, females may lay more eggs of one sex as a result of pre- or post-ovulatory mechanisms of sex allocation (primary sex ratio; Hardy 1997; Komdeur and Pen 2002; Rutkowska and Badyaev 2008). Second, after incubation, the hatchability of eggs can vary in relation to sex (Cichoń et al. 2005). This can result from pre-laying resource allocation processes (i.e. differential female investment in eggs; Cordero et al. 2001; Badyaev et al. 2006) or from sex-specific egg survival due to differential sensitivity to breeding conditions (Cichoń et al. 2005). Third, the survival of nestlings of one sex might be reduced or enhanced due to selective parental care (Droge et al. 1991; Espíndola-Hernández et al. 2017), nestling competition (Anderson et al. 1993; Oddie 2000) or non-random occurrence of sexes in the hatching sequence (Bortolotti 1986; Kilner 1998; Badyaev 2002). Clarifying the roles of the potential proximate mechanisms operating in different developmental stages requires evaluation of the relationships between sex-specific nestling development and survival and sex ratio patterns within broods.

Unequal costs of rearing sons and daughters often result in constraints of optimal parental resource allocation to offspring under unfavourable conditions (Krijgsveld et al. 1998; Hasselquist and Kempenaers 2002). In species with sexually size dimorphic nestlings, there are two contrasting scenarios of nestling survival under unfavourable rearing conditions: First, the individuals of the larger sex show a reduced survival during periods of food limitation due to higher energy requirements ('costly sex hypothesis'; Fiala and Congdon 1983; Weatherhead and Teather 1991; Krijgsveld et al. 1998; Nicolaus et al. 2009). Second, the survival of the smaller sex can be lower because of disadvantages in competition and because of having less reserves in periods of food scarcity ('competitive advantage hypothesis'; Anderson et al. 1993; Arroyo 2002; Hipkiss et al. 2002; Nicolaus et al. 2009).

Within broods, hatching asynchrony leads to a hierarchy in size and competitive ability between siblings (Bortolotti 1986; Uller 2006). Usually, the last hatched nestling (assigned with the highest nestling rank) is the smallest with the highest chance of starvation (e.g. Arroyo 2002; Perrig et al. 2014). As a consequence, the highest rank might be disproportionately affected by sex-specific survival and more strongly biased towards the more robust sex at fledging. However, as a strategy to maximise survival of the entire brood, parents could allocate the larger sex to late-laid eggs to minimise detrimental effects of sibling competition (Bednarz and Hayden 1991; Uller 2006; Penteriani et al. 2010; Gilby et al. 2012). Yet, competitive differences between siblings and susceptibility to harsh conditions may not be linear during nestling development but depend on sex-specific growth patterns (Bortolotti 1986). The combination of sexes among nestling ranks may thus be adjusted in a way that maximises offspring survival in the most sensitive nestling stage, depending on sex-specific growth patterns (Bortolotti 1986). In this study, we use a comprehensive dataset of nestling sex, body weight, hatching rank and

survival to investigate nestling sex ratio (hereafter ‘NSR’, defined as proportion of males) bias in little owls *Athene noctua*. In little owls, two patterns suggest sex-biased nestling survival: First, there is a small but consistent sex dimorphism in adult birds (Females c. 10% heavier than males; Van Nieuwenhuysse et al. 2008). Second, the survival of early-hatched nestlings is disproportionately high, particularly in poor food conditions (Perrig et al. 2014). However, although an NSR bias might have consequences for population dynamics of this endangered species, neither the existence nor the underlying mechanisms of an NSR bias in little owls are known. Thus, we aimed at (1) quantifying nestling sex dimorphism and the associated sex-related nestling survival to assess if the ‘costly sex hypothesis’ or the ‘competitive advantage hypothesis’ applies, and (2) investigating sex ratio patterns within and between broods to investigate potential pre- and post-hatching mechanisms causing biased NSR. So far, few studies have investigated both, sex-specific survival and parental sex allocation by analysing sex-dimorphism, nestling survival and sex-ratio patterns simultaneously. The exceptionally large sample size used here allows detecting even small deviations from sex ratio parity and reduces the likelihood for spurious findings (Hasselquist and Kempenaers 2002).

## Materials and methods

### Study species

The little owl *Athene noctua* is a resident Eurasian nocturnal raptor which feeds on small mammals, birds, arthropods and earthworms (Van Nieuwenhuysse et al. 2008; Gruebler et al. 2018). The little owl is territorial and monogamous with rare to no extra-pair paternity (Hurst 2009). One to seven eggs (with an average clutch size from 2.65 to 5.24 in Europe) are laid in cavities (mainly nest boxes in central Europe) with incubation starting variably between the first egg, subsequent eggs or even not before the clutch is complete (Van Nieuwenhuysse et al. 2008; van Harxen et al. 2018). Accordingly, after incubation, some of the eggs hatch at the same day whereas some hatch at 1-day intervals (van Harxen et al. 2018). Little owl nestlings become ready to fledge for the first time at an age of 28 to 40 days but parents continue to feed the owlets for about 1 month after fledging (Van Nieuwenhuysse et al. 2008). Survival rate of nestling little owls from hatching to fledging ranges from 27 to 86% (Van Nieuwenhuysse et al. 2008; Thorup et al. 2010; Perrig et al. 2017). Though adults of the two sexes show overlap in body weight (females 170–250 g; males 160–240 g), average body weight of females is higher than that of males (females 181 g; males 164 g;

Van Nieuwenhuysse et al. 2008). In the study area, many broods are raised under suboptimal conditions mainly limited by food availability (Thorup et al. 2010; Perrig et al. 2014, 2017; Michel et al. 2017; Gruebler et al. 2018).

### Data collection

We studied 307 little owl broods (846 individuals) from 2009 to 2013 in the German county of Ludwigsburg, Baden-Württemberg (see Perrig et al. 2014) and six additional areas of Germany, Netherlands and Denmark in 2013 (resulting in a total of 7 study areas; Table A1). Between early April and mid-July, nest boxes were checked monthly to look for signs of occupation. If occupation seemed likely, nest boxes were inspected every week to register clutch sizes. We ringed nestlings in all study areas at an age of  $20.0 \pm 0.2$  days (mean  $\pm$  1 SE,  $N = 774$  individuals with known age; hereafter: “sampling period: ringing”). We weighed nestlings with an electronic balance to the nearest 0.1 g and measured the length of the 8th and 9th primary feather with a calliper to the nearest 0.1 mm. Finally, we sampled 3 to 5 growing breast feathers for genetic sex determination. To investigate survival, we visited 187 broods again at an age of  $30.3 \pm 0.2$  days (mean  $\pm$  1 SE,  $N = 187$ ; hereafter “sampling period: second check”) and recorded the remaining individuals, assuming that missing individuals had died.

We assessed the individual hatching rank of nestlings (hereafter “rank”) within broods using the length of primary 9 or primary 8 feathers. As the lengths of primary 9 (model estimate  $-0.969$ ; 95% credibility interval  $-3.432$  to  $1.548$ ) and primary 8 (model estimate  $-1.058$ ; 95% credibility interval  $-3.904$  to  $1.878$ ) do not differ between the sexes, they represent reliable traits for assessing nestling ranks. For the nestlings in the Netherlands (Table A1), we used wing length to determine nestling rank instead, as no feather length was measured. The largest nestling in each brood based on feather or wing length was assigned with rank one. We calculated brood age (age of the first-hatched nestling in a brood) and individual age by using the relationship between age and primary length (Juillard 1979). This calculated age was verified by the age estimates taken at the first visit after hatching. Although little owls do not always hatch in a clearly staggered pattern and mothers may be able to adjust egg size in addition to laying sequence, hatching order is often highly related to laying order and generally has the strongest effect on developmental differences after hatching—even in simultaneously hatching species (Magrath 1990; Bollinger 1994; Clotfelter et al. 2003). We thus assume that size differences at ringing represent hatching order (and thus, laying order), even if nestlings hatched with intervals smaller than a day. Because the little owl broods were ringed at different age, we used the residuals for body weight from a mean growth curve based on all individuals of the Ludwigsburg area (Perrig et al. 2017). Individual age was only used for calculating weight residuals, i.e. age-corrected comparable weight measures of nestlings.

Only brood age entered the statistical analyses. The only brood with a brood size of six was excluded from the analyses as no statistical estimation was possible with a sample size of one. As body weight and rank were not determined for all individuals that were sexed, we used a subset of 774 individuals from 288 broods for sex dimorphism evaluations. For the evaluation of NSR patterns only families with all sexes and ranks known were used (762 individuals from 282 broods at ringing and 404 individuals from 172 broods at the second check). Finally, the evaluations of survival before ringing were based on the 175 broods with known clutch size (mean  $\pm$  1 SE =  $3.54 \pm 0.07$  eggs; min = 1; max = 7).

### Sex determination

Genetic sex determination based on feather samples were done by IDEXX GmbH in Ludwigsburg, Germany in the years 2009 to 2012 (138 feather samples), by J. Hurst at the Albert-Ludwigs-University Freiburg in 2009 (39 feather samples; see Hurst 2009) and by LABOKlin Labor für Klinische Diagnostik GmbH & Co. KG in Bad Kissingen, Germany for the year 2013 (669 feather samples). Repeated sex determinations based on two feather samples of 40 individuals revealed 100% agreement.

It was not possible to record data blind because our study involved focal animals in the field.

### Statistical analyses

All statistical analyses were done in R v. 3.4.3 (R Core Team 2017). Full models based on a priori hypotheses were constructed and Bayesian posterior distributions simulated (5000 simulations) with the R package ‘arm’ (Gelman A, Hill J 2007) for deriving model estimates, 95% credibility intervals (95% CrI) and model predictions. Non-significant interaction terms were eliminated stepwise based on the Bayesian Information Criterion (BIC; Burnham and Anderson 2002), but no model selection was performed on main effects. All numerical explanatory variables were standardised prior to the analyses to get a predictor with mean of zero and standard deviation of one and model assumptions were tested visually following Zuur et al. (2013) and Komer-Nievergelt et al. (2015).

### NSR patterns

To analyse the NSR of little owl nestlings, we fitted generalised linear mixed-effects models (GLMMs) with binomial error distribution (logit-link function) in the R package lme4 (Bates et al. 2015). We modelled the nestling sex ratio for the data at ringing and the second check separately, using the sex of individual nestlings as binary response and nestling rank (factor with 5 levels) and brood size (numeric) as explanatory variables. A random factor with levels for each combination of sampling area

and year (hereafter ‘areayear’; 11 levels) was created to account for the non-nested nature of sampling area and year. In addition, brood identity (hereafter ‘brood id’) nested in areayear was included as random factor to account for multiple nestlings within broods. To approximate the primary sex ratio, we additionally modelled the subset of the broods with no mortality until ringing ( $N = 291$  individuals from 89 broods) using the same model structure as for the full dataset.

### Temporal patterns in NSR and brood size

We analysed the temporal changes in overall brood sex ratios by fitting a GLMM with binomial error distribution (logit-link function) with the number of males among the number of chicks as binomial response variable, and hatching date, brood age and brood size as explanatory variables. As we used the measurements from both sampling occasions in the same model, we included brood id nested in areayear and areayear as random factors. In addition, we modelled the temporal changes in brood size at ringing by fitting a GLMM with Poisson error distribution (log-link function) with hatching date and brood age as fixed explanatory variables. Brood id nested in areayear and areayear were included as random factors to account for the repetition in measurements from both sampling occasions.

### Sexual weight dimorphism

Overall body weight differences between male and female nestlings were evaluated using linear mixed-effects models (LMMs; package lme4; Bates et al. 2015) with weight residuals as response, sex (binary) as fixed and areayear as well as brood id nested in areayear as random factors. To model the sex dimorphism patterns across nestling ranks, we additionally included nestling rank and the interaction between rank and sex as well as the numerical control covariates brood size, hatching date and brood age in these models.

### Nestling survival

To determine the survival of nestlings from hatching to ringing, we fitted a GLMM with binomial error distribution (logit-link function) and an intercept only with the binomial response variable (brood size at ringing/number of dead individuals before ringing). To evaluate the factors affecting nestling survival before ringing, we included the numerical explanatory variables hatching date, brood age and clutch size in a second model. We had to include brood id to account for overdispersion at the brood level and therefore also used areayear and brood id nested in areayear as random factors here. Sex-specific survival before ringing could not be analysed because sex determination was only done for nestlings still living at ringing.

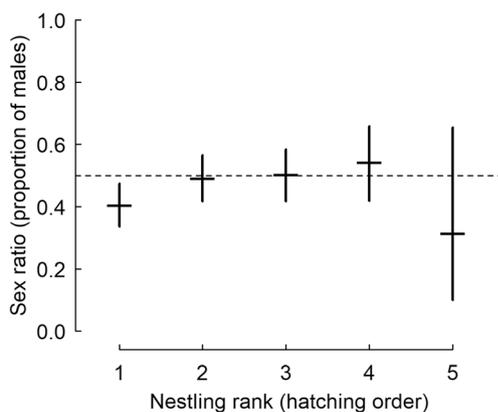
To investigate the survival of nestlings between ringing and the second check, we analysed the binary variable nestling dead or alive at the second check (0/1) in a GLM (logit-link function) with nestling rank, sex, nestling body weight and the quadratic polynomial of body weight, as well as the numerical covariates brood size, hatching date, brood age and time between the nest visits as fixed factors. In addition, we tested the interactive effect of rank and sex, rank and body weight, sex and body weight and sex and hatching date. We used GLM, since mixed models using lme4 (Bates et al. 2015), glmmTMB (Brooks et al. 2017) and glmmPQL (Venables and Ripley 2002) all failed to reliably estimate model parameters when brood id was included as a random factor. The reason was that having many broods with all 1 values (no losses) led to strong overestimation of survival in all cases with 95% credibility intervals of predictions not incorporating raw data means. A binomial model fitted to the number of dead and alive nestlings per brood had an overdispersion coefficient of 1.36. This showed that the between-brood variance in the data is not extremely high. Therefore, we assume that ignoring the non-independence of nestlings from the same brood in the model had a negligible effect on the results.

**Data availability** The datasets analysed during the current study are available from the corresponding author on reasonable request.

## Results

### Nestling sex ratio patterns

The overall NSR of little owls was 0.473 (proportion of males) at ringing and 0.453 at the second check. Within broods, the



**Fig. 1** Nestling sex ratio patterns of little owl nestlings among nestling ranks at ringing (model predictions; means  $\pm$  95% CrI;  $N = 762$  individuals from 282 broods). Sample sizes were  $N = 281$  individuals for rank 1,  $N = 234$  individuals for rank 2,  $N = 164$  individuals for rank 3,  $N = 73$  individuals for rank 4 and  $N = 10$  individuals for rank 5. The effect of brood size was set to its mean value

**Table 1** Factors affecting the nestling sex ratio at ringing

Fixed variables	Estimate	95% CrI
Intercept	-0.382	-0.657 to -0.110
Rank 2	0.345	-0.010 to 0.703
Rank 3	0.392	0.007 to 0.801
Rank 4	0.552	-0.020 to 1.114
Rank 5	-0.392	-1.848 to 1.063
Brood size	-0.081	-0.262 to 0.098
Random effects	SD	
Brood id	0.402	
Areayear	0.174	

Parameter estimates and 95% CrI from the GLMM with binary error distribution modelling individual nestling sex (probability to be a male) are shown, as well as the standard deviation (SD) of the random effects. The intercept represents rank 1 with average brood size, and the other parameter estimates the deviation from the intercept. Brood size was standardised before entering the model. Effects with CrI not overlapping zero are printed in italics.  $N = 762$  individuals from 282 broods

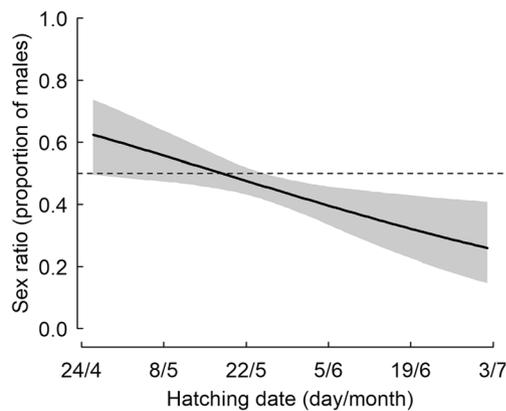
NSR was female biased on rank 1 but not on ranks 2 to 5 (Fig. 1; Table 1). Controlling for the rank effect, we found no additional effect of brood size on NSR, and thus, the pattern of female bias on rank 1 was comparable across all brood sizes (Table 1; Fig. A1). Accordingly, small broods showed a higher NSR bias than large broods because rank 1 contributed more to the brood NSR. The observed effects were persistent to the second check and the bias in rank 1 even reinforced (mean 0.369, 95% CrI 0.293 to 0.453, Fig. A2, Table A2). In the subset of broods where no mortality occurred until ringing, there were no significant biases or effects of brood size (Table A3). However, although not significant due to low sample size, the distinct pattern with a female-biased first rank also occurred in these broods, suggesting that it is already present at hatching (Table A3).

### Temporal patterns in nestling sex ratios

The overall sex ratio decreased with hatching date and reached a significant female bias in later broods (hatched from beginning of June; estimate -0.238; 95% CrI -0.410 to -0.065; Fig. 2). Furthermore, the overall sex ratio tended to decrease with brood age, yet with CrI overlapping zero (estimate -0.108; 95% CrI -0.251 to 0.029). In contrast, brood size had no effect on the overall sex ratio (estimate -0.034; 95% CrI -0.205 to 0.131). Brood size itself decreased with hatching date (estimate -0.081; 95% CrI -0.139 to -0.026) and age of the brood (estimate -0.116; 95% CrI -0.172 to -0.060).

### Sexual weight dimorphism of nestlings

At ringing, female nestlings were generally 3.02 g (95% CrI 1.33 to 4.73) heavier than male nestlings. This represents a small (2.6%) but consistent weight difference when compared



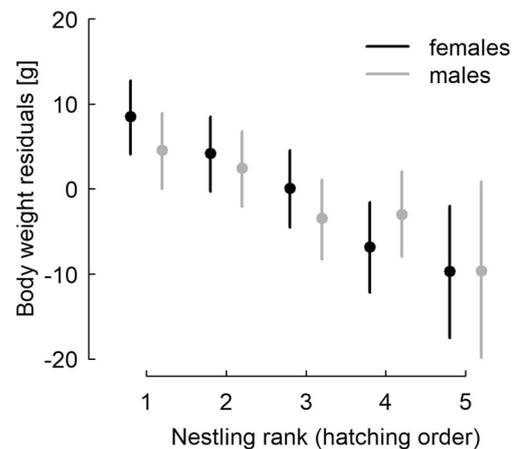
**Fig. 2** Temporal patterns in overall nestling sex ratios. Overall sex ratio in broods in response to hatching date based on 488 brood visits (307 broods at ringing and 181 at the second check). The solid line shows the mean value and grey shaded areas 95% CrI of model predictions with all other model predictors set to their mean values

with the average nestling weight of 115.29 g at ringing. Yet, this effect varied across ranks (interaction of sex and rank; Table 2; Fig. 3) and nestling body weight decreased with rank (Table 2; Fig. 3). Brood size, hatching date and brood age were not important predictors of body weight residuals (Table 2), and broods of different sizes therefore showed body weight patterns similar to the pattern of all brood sizes in combination (Fig. A3). Consequently, chicks of small broods had a higher average body weight compared with those of large broods.

**Table 2** Factors affecting body weight in little owl nestlings

Fixed variables	Estimate	95% CrI
Intercept	8.527	4.298 to 12.737
Sex	- 3.969	- 6.774 to - 1.346
Rank2	- 4.359	- 6.630 to - 2.040
Rank3	- 8.464	- 11.170 to - 5.886
Rank4	- 15.311	- 19.112 to - 11.542
Rank5	- 18.218	- 25.069 to - 11.076
Sex × rank 2	2.260	- 1.436 to 6.023
Sex × rank 3	0.423	- 3.501 to 4.518
Sex × rank 4	7.713	2.353 to 13.007
Sex × rank 5	4.018	- 7.827 to 16.107
Brood size	0.246	- 1.516 to 2.033
Hatching date	- 0.040	- 2.026 to 1.892
Brood age	- 0.431	- 2.293 to 1.438
Random effects	SD	
Brood id	14.165	
Areayear	6.160	

Parameter estimates and 95% CrI from the LMM modelling individual nestling weight residuals (in g) are shown, as well as the standard deviation (SD) of the random effects. The intercept represents females with rank 1 and average values for all numerical predictors, and the other parameter estimates the deviation from the intercept. Numerical predictors were standardised before entering the model. Effects with CrI not overlapping zero are printed in italics.  $N = 774$  from 288 broods



**Fig. 3** Sexual weight dimorphism patterns among nestling ranks at ringing. Model prediction means  $\pm$  95% CrI are shown ( $N = 774$  individuals from 288 broods). The  $y$ -axis denotes the body weight residuals corrected for nestling age. All other model predictors were set to their mean values

### Nestling survival

The average nestling survival from hatching to ringing (only considering broods that survived to ringing) was  $\Phi = 0.842$ . This probability decreased with increasing clutch size (estimate - 0.311; 95% CrI - 0.567 to - 0.052) but was not affected by hatching date (estimate - 0.189; 95% CrI - 0.445 to 0.064) or brood age at ringing (estimate - 0.095; 95% CrI - 0.363 to 0.175). The overall probability of surviving from ringing to the second check was  $\Phi = 0.819$ . Survival of both sexes increased with nestling body weight in a saturating curve (Table 3; Fig. 4a). In addition to the weight effect, the survival of males was c. 6% below female survival (Table 3; Fig. 4a, b). Nestlings of large broods showed significantly reduced survival (Table 3) but neither hatching date nor brood age or the time between nest visits was associated with nestling survival probability (Table 3). Nestling survival tended to decrease with increasing rank, but with 95% CrI overlapping zero (Table 3; Fig. 4b). The insignificant differences are partially explained by the correction for body mass, as survival for rank 4 differed significantly from rank 1 (estimate - 0.782; 95% CrI - 1.525 to - 0.002), if body mass was not included in the model. Finally, there was no significant interactive effect between rank and sex, rank and body weight, sex and body weight and sex and hatching date.

### Discussion

We show that NSR in little owls is female-biased and that this bias varies with nestling rank. First-hatched nestlings were more likely to be females, while higher nestling ranks were unbiased. In addition, NSR varied with hatching date with

**Table 3** Factors affecting nestling survival from ringing to fledging

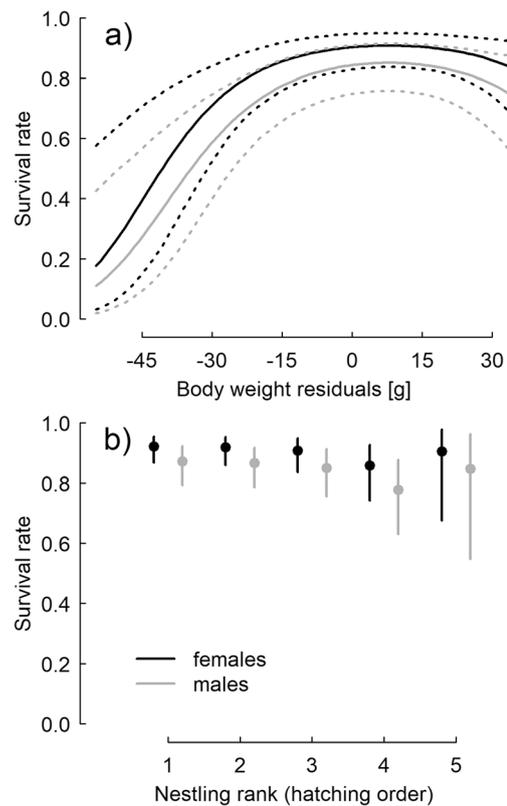
Fixed variables	Estimate	95% CrI
Intercept	2.472	1.893 to 3.028
Sex	-0.550	-1.028 to -0.071
Rank 2	-0.042	-0.697 to 0.612
Rank 3	-0.179	-0.874 to 0.514
Rank 4	-0.661	-1.452 to 0.100
Rank 5	-0.205	-1.700 to 1.369
Body weight	0.105	-0.146 to 0.363
Body weight <sup>2</sup>	-0.299	-0.482 to -0.117
Brood size	-0.674	-1.005 to -0.340
Hatching date	0.062	-0.233 to 0.351
Brood age	-0.029	-0.475 to 0.415
Time between nest visits	-0.240	-0.653 to 0.158

Parameter estimates and 95% CrI from the GLM with binary error distribution modelling individual survival (nestling dead or alive at the second check) are shown. The intercept represents females with rank 1 and average values for all numerical predictors, and the other parameter estimates the deviation from the intercept. Numerical predictors were standardised before entering the model. Effects with CrI not overlapping zero are printed in italics.  $N = 515$  individuals from 187 broods

broods becoming more female-biased as the breeding season progressed. Females were heavier than males and had higher survival, supporting the competitive advantage hypothesis. While this sex-specific survival can explain a general NSR bias, it cannot explain the sex ratio pattern among ranks and the NSR variation across the season. As body mass and survival decreased with nestling rank, sex-specific survival rather affected higher ranks than first-hatched nestlings. In combination, our results suggest that complex interactions between both, sex-biased nestling survival and pre-hatching sex allocation mechanisms affect NSR at fledging.

The nestling weights at ringing reflect the adult sex dimorphism in little owls with slightly but consistently heavier females than males (Van Nieuwenhuyse et al. 2008). These differences can arise from larger egg size (Mead et al. 1987), faster early development (Teather and Weatherhead 1994) and/or higher parental investment into female nestlings compared with males during the nestling period (Droge et al. 1991). On top of that, potential competitive benefits of females in aggressive interactions between nestlings are likely to reinforce existing differences (Anderson et al. 1993; Oddie 2000; Hipkiss et al. 2002). Even in broods with nearly synchronous hatching, small differences can be reinforced considerably by these mechanisms.

Light nestlings had lower survival than heavy nestlings, corroborating the importance of nestling weight for survival and providing support to the competitive advantage hypothesis (e.g. Magrath 1991; Nicolaus et al. 2009; Perrig et al. 2014, 2017). This suggests that the mechanisms affecting weight are also the indirect drivers of weight-specific survival. Even in the absence of sibling aggression, as soon as a size



**Fig. 4** Model predictions of survival rates of female and male nestlings between ringing and the second check in response to **a** nestling body weight (solid lines = means; dotted lines = 95% CrI) and **b** nestling rank (points = means; error bars = 95% CrI) based on  $N = 515$  individuals from 187 broods. The  $x$ -axis in **a** denotes the body weight residuals corrected for nestling age in grams. All other model predictors were set to their mean values. Note that the effects shown are corrected for the other covariates included in the model (Table 3). For example, the effect of rank is corrected for body weight and vice versa

dimorphism occurs, males are more likely to die under food limitation due to fewer fat reserves (Arroyo 2002). In addition, the fact that males showed a lower survival probability also when accounting for body weight suggests that males are generally more sensitive to harsh environmental conditions than females or suffer more from sibling competition (cf. Fletcher and Hamer 2004; Cichoń et al. 2005).

The mechanisms affecting sex-specific survival can explain the development of an overall female bias within little owl broods and are supported by the increase in the NSR bias during the nestling period. However, the consistent pattern of a biased NSR in first eggs is highly unlikely to be the result of sex-specific survival alone and thus suggests that the bias already exists at hatching. Sex-specific survival on its own would affect higher nestling ranks more than low ranks, as nestling weight and consequently survival decreases from low to higher nestling ranks (cf. Hasselquist and Kempenaers 2002). Although surprising and rarely observed, similar patterns with a bias in first chicks but unbiased rest of the brood were found in

birds before (for example in Harris' hawks *Parabuteo unicinctus*; Bednarz and Hayden 1991). A likely explanation for this pattern is post-ovulatory selection of the sex of the first egg in a line with later eggs left to chance (Emlen 1997; Komdeur and Pen 2002). Alternatively, a faster embryonic development of females can result in a higher probability to hatch first and gain competitive advantages (Kilner 1998; Blanco et al. 2003; Uller 2006). Pre-ovulation adjustments on the other hand would more likely affect all ranks comparably (Emlen 1997) and therefore seem unlikely in our case. The fact that decreasing NSR across the season could not be explained with sex-specific survival and only partially with decreasing brood sizes provides additional support for pre-hatching mechanisms. In addition, it suggests parental sex allocation strategies rather than sex-specific embryonic development, as seasonal changes in embryonic development are unlikely.

The evolution of post-ovulatory sex allocation strategies or sex-specific embryonic development predicts that parents should have higher benefits from offspring of one sex (here females) than from the other (Hasselquist and Kempenaers 2002; Komdeur and Pen 2002). This will be particularly true in adverse breeding conditions when clutch sizes are small and nestling survival is low and consequently the sex ratio bias is higher due to the stronger influence of the first rank. This is supported by the observed decreasing NSR across the season, when breeding conditions become worse. There are at least four non-exclusive ultimate mechanisms resulting in a condition-dependent female-biased primary sex ratio with a stronger bias on rank one: (1) females disperse farther (Van Nieuwenhuysse et al. 2008) and may thus provide fitness benefits through the higher probability of escaping suboptimal local conditions (Julliard 2000; Hjernquist et al. 2009). (2) Adult female little owls show lower year-to-year survival than adult males under suboptimal conditions (likely due to higher costs of breeding; Michel 2016), as it is commonly observed in bird species (Donald 2007). Thus, poor conditions may result in a male-biased breeding population (reaching a predicted sex ratio of 0.57 after 1 year; Michel 2016) providing higher fitness benefits to parents with daughters due to their higher likelihood of reproduction (Fisher 1930; Donald 2007). Allocating the desired sex ratio bias to rank 1 might be explained by post-ovulatory selection of the sex of the first egg (Emlen 1997; Komdeur and Pen 2002) and increases the chances that the bias persists into the fledgling population. This is again particularly true for adverse conditions, as the strength of the overall bias in the fledgling population will be stronger due to lower survival and smaller average brood size than under good conditions (Michel et al. 2017). While the first two mechanisms explain fitness effects through differential reproductive output, the two remaining explanations relate

to optimal parental resource allocation during the breeding season: (3) In contrast to adjustments to reduce intra-brood competition, where the smaller sex should be favoured on lower ranks, placing the larger (i.e. more expensive) sex on lower ranks could be a strategy to minimise energetic costs of nestling mortality (Penteriani et al. 2010). Alternatively, growth patterns of siblings may be sex-specific and depend on sibling sex as shown by Bortolotti 1986. Thus, relative size differences and associated competitive differences between siblings during the most sensitive nestling stages may not be reflected by size differences measured at ringing. Finally, (4) if females profit more than males from being on low ranks, for example through greater benefits from higher amounts of provided food, placing females on lower ranks will be beneficial (Badyaev 2002; Carranza 2004; Uller 2006). In line with these predictions, a shift in the NSR across the season may be a response to deteriorating environmental conditions (e.g. decreasing vole availability or accessibility; Apolloni et al. 2018) or to differential fitness prospects of the sexes in response to the time of fledging (Dijkstra et al. 1990; Daan et al. 1996; Neto et al. 2011). Males may suffer more from a delayed start of breeding in the next year or the decreased probability of breeding as yearling, leading to reduced fitness prospects of late fledging males compared to late fledging females (Dijkstra et al. 1990; Daan et al. 1996; Cordero et al. 2001).

## Conclusions

The overall NSR bias of little owls suggests unequal costs of and/or unequal fitness returns from raising sons and daughters. The observed patterns strongly suggest that both, sex-specific survival and parental sex allocation strategies are involved and that both are context-dependent. Thus, NSR at fledging most probably depends on complex interactions between the two mechanisms. Yet, so far, most studies have considered only one of the two mechanisms to explain biased sex ratios and may have missed the context-dependent involvement of both mechanisms. Our results also suggest that the varying bias in NSR in little owls is most likely both, a consequence of—and an adaptation to—suboptimal breeding conditions. This is underlined by the fact that NSR was unbiased in the Dutch study area that seems to offer more favourable conditions (e.g. highest body weight of nestlings; high average brood sizes; Table A4) than other study regions (see also van Harxen et al. 2018) and tended even to be male-biased in the area with the highest average brood size (Table A4). Thus, we propose an adaptive feedback cycle driven by varying effects of breeding conditions on adult sex ratio resulting in an adjustment of NSR. Imperfect feedback cycles will lead

to skews in adult sex ratios which can have significant effects on social behaviour (Michler et al. 2011; Eberhart-Phillips et al. 2018) and population dynamics (Donald 2007) and seems to be more common in threatened bird species (Donald 2007) and small populations (Morrison et al. 2016).

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### Compliance with ethical standards

**Ethical approval** Handling and ringing of little owl nestlings in Germany was carried out under the permit of the regional council of Baden-Württemberg, Germany (licence No. 35e9185.81/0288), the regional council of Rheinland Pfalz (licence Az 42/553-253) and Stuttgart (licence Az 55-8853.17), the Struktur- und Genehmigungsdirektion (SGD) Süd and SGD Nord of Rheinland-Pfalz, as well as Vogelwarte Radolfzell (licences no. 1146, 1191; 1403 and 1903). Handling and ringing of the nestlings in Denmark was carried out under licence from Copenhagen Bird Ringing Centre (A-392 personal ringing licence to LBJ). The sampling of 5 growing breast feathers in Denmark was permitted by The Animal Experiments Inspectorate (#2011/561-17). Handling and ringing in the Netherlands was carried out under the licence from Vogeltrekstation, Dutch centre for avian migration and demography (R. van Harxen-848). The sampling of growing breast feathers in the Netherlands was permitted by Dierexperimentencommissie Koninklijke Academie van Wetenschappen/NIOO 13. 07 advies. All procedures followed the ASAB/ABS guidelines for the ethical treatment of animals in behavioural research and teaching and all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The handling of birds was performed with maximum care and disturbance to nests kept to a minimum. Ethical approval for involving animals in this study was received through the application procedure for ringing permits and the scientific commission of the Swiss Ornithological Institute.

**Conflict of interest** The authors declare that they have no conflicts of interest.

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