Fledgling sex-ratio is biased towards the helping sex in a Neotropical cooperative breeder, the brown-and-yellow marshbird (*Pseudoleistes virescens*)

Myriam E. Mermoz [a,b,*], **Cecilia Villarruel** [b,c], **Alicia de la Colina** [a,d] and **Bettina Mahler** [a,b]

[a] Instituto de Ecología, Genética, y Evolución de Buenos Aires (UBA-CONICET), Intendente Güiraldes 2160 — Ciudad Universitaria — C1428EGA, CABA, Argentina
[b] Departamento de Ecología, Genética, y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160 — C1428EGA Ciudad Universitaria, CABA, Argentina
[c] Current address: Instituto de Fisiología, Biología Molecular y Neurociencias (UBA-CONICET), Intendente Güiraldes 2160 — C1428EGA Ciudad Universitaria, CABA, Argentina
[d] Current address: Departamento de Conservación e Investigación, Fundación Temaikèn — B1625 Escobar, Prov. de Buenos Aires, Argentina
[*] Corresponding author's e-mail address: myriammermoz@gmail.com

Abstract

In many cooperatively breeding species, helpers increase the breeding success of their parents. The repayment hypothesis predicts a skewed sex-ratio towards the helping sex at population level; at individual level bias would increase in broods attended by a smaller number of helpers. We studied a brown-and-yellow marshbird (*Pseudoleistes virescens*) population during 11 breeding seasons. We found that 90% of helpers were males and that they increased nestling survival, although this effect disappeared in presence of parasitic shiny cowbirds. Helpers sometimes helped at nests of adults other than their parents. Population sex-ratio of fledglings was highly skewed towards males (1.4:1). At individual level, male-biased sex-ratio of fledglings was more pronounced early in the season and increased with brood losses but was not affected by number of helpers. Marshbirds feed at communal areas so retaining helpers would not be costly. Therefore, a general skew towards males might be the best adaptive strategy.

Resumen

En numerosas especies con cría cooperativa, los ayudantes aumentan el éxito reproductivo de sus padres. La hipótesis del reembolso predice una relación de sexos sesgada hacia el sexo que ayuda a nivel poblacional; a nivel individual, el sesgo sería más pronunciado en aquellas nidadas atendidas por menos ayudantes. Estudiamos una población de *Pseudoleistes virescens* durante 11 temporadas reproductivas. Encontramos que el 90% de los ayudantes eran machos y que aumentaron la super-vivencia de los pichones en ausencia de parásitos de cría *Molothrus bonariensis* en el nido. Los ayudantes a veces ayudaron en nidos de adultos que no eran sus padres. La relación de sexos en la población de volantones estuvo fuertemente sesgada hacia machos (1.4:1). A nivel individual, la relación de sexos de los volantones estuvo más sesgada hacia machos al inicio de la temporada reproductiva y aumentó con las pérdidas dentro de la nidada, pero no varió con el número de ayu-dantes. Los *P. virescens* se alimentan en áreas comunales, por lo que retener ayudantes no sería costoso. Consecuentemente, generalizar en el sesgo hacia machos podría ser la mejor estrategia adaptativa.

1. Introduction

In species with cooperative breeding, reproductive units are composed of more than two individuals that cooperate to rear a single brood (Brown, 1987; Clutton-Brock, 2002). The term 'cooperative breeding' describes a variety of breeding systems that share an unequally divided reproduction among group members, with some subordinate individuals obtaining only a small portion of the reproduction or none at all (Cockburn, 1998; Cockburn & Russell, 2011; Lukas & Clutton-Brock, 2012; Koenig et al., 2016; Griesser et al., 2017). In most cooperatively breeding birds, the breeding pair is assisted by non-breeding helpers that are generally previous offspring or close rela-tives (Ligon & Burt, 2004; Griesser et al., 2017). Various hypotheses based on ecological constraints for dispersal, benefits of family living, and the effect of both stable and unpredictable environments, have been proposed to explain why helpers delay dispersal and do not breed independently (Ruben-stein & Lovette, 2007; Jetz & Rubenstein, 2011; Koenig, 2017; Shen et al., 2017). In birds, females are usually the dispersing sex and males are more philopatric (Greenwood, 1980; Mabry et al., 2013). Hence, it is not surpris-ing that helpers are predominantly males among cooperatively breeding birds (Ligon & Burt, 2004; Koenig et al., 2016; Komdeur et al., 2017). Neverthe-less, in some species helpers are of both sexes (Covas et al., 2008; Keynan & Ridley, 2016; Napper & Hatchwell, 2016; Ridley, 2016; Sorato et al., 2016; Nelson-Flower et al., 2018) or are predominately females (Komdeur et al., 1997; Berg, 2004).

The impact that helpers have on the reproductive pair's breeding success varies among species (Brown, 1987; Cockburn, 1998; Koenig et al., 2016). Moreover, within the same species, helpers might or might not benefit recipients, depending on external conditions (Wright & Russell, 2008; Cockburn & Russel, 2011; Griesser et al., 2017). For example, in the Seychelles warbler (*Acrocephalus sechellensis*) helpers increase the fitness of their parents when inhabiting good-quality territories, whereas in low-quality territories helpers undermine their parents' breeding success and are not tolerated (Komdeur et al., 2016). Similarly, in the acorn woodpecker (*Melanerpes formicivorus*), the effect of helpers on the recipient's fitness increases in years with higher acorn production (Koenig et al., 2011).

The term sex-ratio refers to the proportion of males to females and it has been proposed that offspring sex-ratio affects parental fitness (Fisher, 1930). If there is an equal cost-benefit relation of producing sons or daughters, there should be an equal proportion of sexes due to a reproductive advantage of the less frequent sex (Fisher, 1930; Hamilton, 1967; Williams, 1979). In contrast, when one sex is more costly to produce or has greater variance in fitness, natural selection will favour those parents who can bias the sex-ratio of their offspring towards the more profitable sex (Trivers & Willard, 1973; Komdeur, 2012). In birds, females might modify the primary sex-ratio of their brood (i.e., sex-ratio at laying) by controlling their offspring's sex before and/or after fertilization (reviewed by Pike & Petrie, 2003; Alonso Álvarez, 2006). The secondary sex-ratio (i.e., brood sex ratio after hatching) might also be biased by differential survival of sexes until fledging age (Szczys et al., 2001; Moreno-Rueda et al., 2014).

In sexually dimorphic species, the larger sex likely demands more energy and resources during development, which can lead to a skewed sex-ratio towards the smaller sex (Sheldon et.al., 1998; Benito & González-Solis, 2007; Juola & Dearborn, 2007; English et al., 2014; Moreno-Rueda et al., 2014). In addition, different factors such as social system, resource availability, date within the breeding season, or parents' characteristics may influence the relative advantage for mothers of producing sons or daughters (Trivers & Willard, 1973; Smallwood & Smallwood, 1998; Juola & Dearnborn, 2007; Komdeur, 2012; Trnka et al., 2012; McNew et al., 2020). For example, sex-ratio theory states that when food is in short supply mothers should overproduce the sex that is cheaper or that has lower variance in reproductive value

(Trivers & Willard, 1973; Clout et al., 2002; Rubenstein, 2007; Neto et al., 2011).

In species with helpers at the nest, the benefits for females of producing sons or daughters may be influenced by the kind and quality of help each sex provides to their parents. The repayment hypothesis states that if the individuals of one sex increase their parents' lifetime reproductive success through assisting them in offspring care, then this helping-sex will be less costly to produce because helpers will partially compensate the costs of their rearing (Emlen et al., 1986). This hypothesis has two general predictions: at population level, offspring sex-ratio will be biased towards the helping sex (Emlen et al., 1986), and at individual level, brood sex-ratio bias should increase towards the helping sex when parents have few or no helpers (Komdeur et al., 1997). Although results of some studies found a bias in brood sex-ratio according totally or partially with the predictions of the repayment hypothesis (e.g. Komdeur, 1996; Clarke et al., 2002; Ewen et al., 2003; Ribeiro et al., 2007; Woxvold & Magrath, 2008; Canestrari et al., 2012), other studies did not find any bias at all (e.g. Koenig & Dickinson, 1996; Berg, 2004; Cockburn & Double, 2008; Nam et al., 2011; Kingma et al., 2011; Khwaja et al., 2018) or even a sex-ratio bias inverse to that predicted by the hypothesis (Doutrelant et al., 2004). Therefore, evidence has not been conclusive about these predictions in cooperatively breeding birds and repayment might not be a general driver for sex allocation (Khwaja et al., 2017).

In this study, we tested the repayment hypothesis in a neotropical cooperatively breeding passerine, the brown-and-yellow marshbird (*Pseudoleistes virescens*). Males and females are monomorphic in size (∼80 g) and colouration (Orians, 1980), indicating similar costs of producing sons and daughters (Juola & Dearborn, 2007) and thus, equal survival of both sexes is expected related to environmental conditions (Sheldon et al., 1998). We used a long-term data set, collected during eleven breeding seasons, to determine whether helpers had an effect on the breeding pair's current reproductive success and were predominantly of one sex. In previous studies, we found that number of helpers increased total delivery of food to the nest implying an increase in the 'per capita' amount received by each nestling (Mermoz et al., 2008; Mermoz, 2011). Therefore, we predict that nestling survival will increase with the number of helpers attending the brood. Since females are generally the dispersing sex (Greenwood, 1980; Mabry et al., 2013), we predict that

sex-ratio of helpers will be largely biased toward males. Finally, considering that the helping sex will bring more benefits to parental individuals, we evaluated if sex-ratio was biased at population and individual level fitting the predictions of the repayment hypothesis, namely, 1) sex-ratio at the population level will be biased toward the males, and 2) sex-ratio bias toward males at individual level will be inversely related to the number of helpers attending the brood.

2. Material and methods

2.1. Study species

Brown-and-yellow marshbirds (hereafter marshbirds) are socially monogamous with facultative cooperative breeding (Orians, 1980). At our study site, marshbirds are year-round residents and roughly 50% of the nests have 1–5 helpers from egg-laying to the end of the nesting cycle. The modal clutch size is 4 eggs (range 3–6), the incubation period lasts 14 days, and the nestlings stay in the nest for 7–10 days (Mermoz & Reboreda, 1998, 2003). Helpers defend the nest against predators and brood parasites, and feed the incubating female, nestlings, and fledglings (Orians, 1980; Mermoz et al., 2013). Marshbirds have very low nesting success and suffer high rates of brood parasitism by shiny cowbirds (*Molothrus bonariensis*); on average only 13% of nests produce marshbird or shiny cowbird fledglings and 66% of nests are parasitized (Mermoz & Reboreda, 1998, 2003). In addition, in nearly one-fourth of the nests, last-hatched nestlings die due to brood reduction (Duré Ruiz et al., 2008). Parents and helpers continue defending fledglings for more than a month after they leave the nest (M.E. Mermoz & G.J. Fernández, unpublished data).

2.2. Study area

The study was carried out near the town of General Lavalle (36°26′S, 56°25′W) in the province of Buenos Aires, Argentina, during eleven breeding seasons (mid-September–late December), between 2001 and 2011. The study area is within the so-called 'flooding pampas', a flat region with most elevation <4 m above sea level. It includes marshes and humid grasslands with scattered patches of native woodlands (mainly *Celtis ehrenbergiana*) in the higher areas, and it is crossed by many artificial drainage canals. The climate is temperate sub-humid, with mean monthly temperatures of 23°C in

January and 13°C in July, and an average annual rainfall of 1000 mm (Soriano et al., 1992). In this area, marshbirds build their open-cup nests 0.3 to 2.0 m above ground in exotic thistles (*Cynara cardunculus*, *Carduus* spp.), native pampa grasses (*Cortaderia selloana*), black rushes (*Juncus acutus*), cattails (*Typha* sp.) and spartina grasses (*Spartina* spp). Throughout each breeding season, we located marshbird nests by surveying the vegetation in the proximity of an artificial drainage canal, dominated by exotic thistles and native plants, and at the ranch Los Zorzales, which comprises mosaics of natural and implanted pastures used for cattle rearing. We caught as many adult individuals as possible using mist nets, although the openness of the habitat and windy conditions diminished our netting effort. We captured 231 individuals that were ringed with a unique combination of coloured plastic rings and a numbered metal ring. On average, 51% (range 18–73%) of marshbird adults associated to nests analysed in this study could be individualized each year (mean number of nests = 11.8 range: 4–20 nests per year; mean number of adults 2.98 range 2–6 per nest).

2.3. Data collection

2.3.1. General methodology

Nests were geo-positioned with a GPS device (Magellan eXplorist 500, MiTAC Digital Corporation) and tagged with a small coloured flag put randomly at least 15 m apart. Whenever possible, nests were checked every other day. Nest monitoring was less intensive during 2010 and 2011 (once every 4–7 days), so data of those breeding seasons were excluded for the analyses of nest success and nestling survival. We determined clutch initiation date as the Julian date in which the first egg was laid (considering 1 September as day 1). During nest visits, we determined the number and identity of the adults associated to the nest by identifying all individuals defending the nest. Defence behaviours included alarm calls, approaches within 3 m, close passes, and leg strikes. This approach gives a good estimation of birds involved in nest attendance as they engage both in nest defence and offspring feeding (Mermoz et al., 2008; Mermoz, 2011). We also checked the contents of nests and considered that a nest was fully depredated when it lost all eggs or nestlings between two visits, and that it was partially depredated when it lost some eggs or nestlings. Nestlings that died due to brood reduction were generally in poor body condition during the previous visit. We considered that a nest was abandoned when eggs or nestlings were cold

or dead. Finally, a nest was recorded to have reached fledgling stage when it produced marshbird or shiny cowbird fledglings. We recorded the number of eggs that hatched from those that survived to the estimated hatching date (i.e., hatching success) and the number of nestlings fledged from those that hatched (i.e., nestling survival). Nestlings older than 7 days can fledge, so we will refer to nestlings that reached day 7 as fledglings. Seven day-old nestlings were ringed with a unique colour combination (four coloured plus a numeric aluminium leg-ring), and a small amount (20–50 μl) of blood was taken through brachial vein puncture. Blood was collected with a heparinized capillary tube, immediately mixed with lysis buffer (100 mM Tris pH 8, 10 mM NaCl, 100 mM EDTA, 0.5% SDS) and stored at room temperature until analysis.

Although male and female marshbirds are similar in colour and size, we could not rule out completely that food availability modified sex-ratio manipulation. In addition, food availability affects nestling (Wiehn & Korpimäki, 1997; Verboven et al., 2001) as well as juvenile and adult survival (Seward et al., 2013). Since food availability is difficult to estimate from direct observations, because marshbirds do not defend foraging areas and feed mostly on terrestrial arthropods by probing the ground with their beak (Orians, 1980), we considered that humidity in the superficial layer of the ground would facilitate feeding. Therefore, we used total rainfall as a proxy of food availability. Empirical information on the relationship of rain and arthropod abundance is scarce, but a recent work, also in the Southern Hemisphere, found a linear effect (Hidalgo Aranzamendi et al., 2019). Precipitation data were obtained from the nearest weather station, situated in the city of Dolores (60 km northwest of the study area), which is also within the flooding pampas. For nestling survival analysis, we considered rainfall over the 10 days of nestling period plus the 15-day period prior to hatching to account for any lag between rain and invertebrate emergence in grasslands (Barnett & Facey, 2016; i.e., total rainfall over the whole 25-day period). For brood sex-ratio analysis we used rainfall over the period of egg formation, considering that in resident passerines, nutrients for egg formation are acquired from four days before the start of laying up to the end of laying (Perrins, 1994) and sex is determined 1–2 h before ovulation (Rutkowska & Badyaev, 2008). We took the modal clutch size of four eggs and like before, we added rainfall over 15 days prior to egg formation to account for any lag between rain and invertebrate emergence (i.e., total rainfall over the whole 23-day period).

2.3.2. *Nestling survival*

We analysed the effect that helpers had on nestling survival as they increase food delivery to the nest (Mermoz et al., 2008; Mermoz, 2011). We used nests found during 2001–2009 that reached fledgling stage. We excluded those parasitized nests in which no marshbird hatched and nests in which nestling losses were clearly due to partial depredation (i.e., nestlings were found dead and injured at the base of the nest or wounded in the nest). In addition, we excluded broods with single-hatched marshbird nestlings, since we could not assign losses unambiguously to brood reduction or predation. Therefore, our data set included successful nests with at least two marshbird nestlings or with marshbird and shiny cowbird nestlings.

2.3.3. *Sex determination*

DNA was extracted from blood samples using a standard salting-out protocol (Miller et al., 1988) and diagnostic sex-linked alleles were amplified using the P8/P2 primer set (Griffiths et al., 1998). Amplifications were performed in 10 μl reaction volumes using 50–100 ng of DNA template, 0.5 μM forward and reverse primers, 0.25 μM dNTPs, 1.5 mM $MgCl_2$, and 0.15 U *Taq* Polymerase (Invitrogen). Annealing temperatures were set at 50°C and repeated for 30 cycles. PCR products were separated on 3% agarose gels stained with GelRed and visualized under UV light. The presence of one band indicated males (ZZ), whereas two bands indicated females (ZW).

2.3.4. *Statistical analysis*

We tested the effect of helpers on nestling survival, while taking into account potential confounding variables (Wright & Russell, 2008), using generalized linear mixed models (GLMM) with binomial errors and logit link function (Crawley, 2007). Nestling survival (i.e., the proportion of nestlings that fledged per brood) was the binomial response variable and explanatory variables were the number of helpers calculated as the maximum number of adults (ringed or not) observed defending the nest during nest checks minus two ('HELP' discrete), presence of a shiny cowbird nestling in the brood ('SC', dichotomous), the interaction HELP:SC, total rainfall during the 15 days previous to hatching plus the 10 days of chick-rearing stage ('RAIN', continuous), and Julian date of clutch initiation ('DATE', discrete). Group identity and year were included as random factors, the latter to account for random variation of breeding seasons (Zuur et al., 2009).

We analysed if sex-ratio of helpers deviates from 0.5 using a binomial test. In addition, we compared sex-ratio of helpers with the brood sex-ratio

of the population of fledglings (i.e., binomial test with $p =$ sex-ratio in the population of fledglings).

We analysed if brood sex-ratio of fledglings deviates from 0.5 at population level using a binomial test. To analyse deviations of brood sex-ratio at individual level, we fitted binomial GLMM with logit link. The dependent variable was proportion of males in the brood with the following explanatory variables: HELP, RAIN and DATE (as defined above), and brood losses ('BL', continuous), defined sensu lato as the proportion of the initial clutch that was lost due to different causes, including partial predation of eggs or nestlings, hatching failures, and brood reduction (Dyrcz et al., 2004; Moreno-Rueda et al., 2014). Thus, it took a value of 0 in complete broods (i.e., those in which all eggs hatched and all nestlings reached the blood-sampling age of 7 days), and a value $0 < BL < 1$ getting closer to one when an increasing proportion of eggs or nestlings was lost before blood sampling age. By including incomplete broods, we avoid restricting sex-ratio analysis to only more successful females, which could be misleading (Fiala, 1980; Krackow & Neuhäuser, 2008). Female identity and year were included as random factors.

GLMMs were fitted using lme4 and MuMIn packages in R software, version 3.5.3 (R Development Core Team, 2019). Models were evaluated using multimodel inference procedures (MMI, Burnham & Anderson, 2002). Our candidate model sets included all possible combinations of predictor variables plus the null model including only the random effects. In the analysis of nestling survival, the interaction term HELP:SC was always included with its main effects. Therefore, our model set included 20 models for nestling survival and 16 for sex-ratio analysis. To check assumptions, we evaluated the global model of each analysis for any pattern in residuals, and estimated zero inflation and overdispersion with the packages DHARMa (Hartig, 2020) and RVAideMemoire (Hervé, 2020). Dispersion parameters did not differ from 1 (nestling survival $\hat{c} = 0.69$ dispersion test $p = 0.176$; brood sex-ratio $\hat{c} = 1.25$ dispersion test $p = 0.864$). Therefore, models were compared using Akaike's information criterion corrected for small sample sizes (AIC_c), so that the best supported model has the lowest AIC_c. The Akaike weight of each model (w_i) represents the probability that it is the best among the candidate models considered. To account for uncertainty in model selection, parameter estimates for each data set were obtained by averaging over more probable candidate models (i.e., those models with a $\Delta AIC_c < 4$ ($\Delta AIC_c =$

AIC_c of focal model — AIC_c best model)). Finally, the 95% confidence intervals (CI) were calculated for every parameter estimate.

3. Results

3.1. Nestling survival

Between 2001 and 2009 we found 660 nests (95 nests in 2001, 56 in 2002, 52 in 2003, 79 in 2004, 26 in 2005, 65 in 2006, 104 in 2007, 154 in 2008 and 28 in 2009). For 619 nests with known fate, 134 reached fledgling stage by producing either marshbird or shiny cowbird fledglings, 474 were depredated (382 during egg stage and 92 during nestling stage), and 11 were abandoned (8 due to egg-pecking by shiny cowbird, 2 due to adverse weather conditions, and 1 due to cattle disturbance). Marshbirds fledged their complete clutch in only 22 of the 134 nests that reached fledgling stage (16.4%). In 102 nests (72.8%), they fledged incomplete clutches due to partial depredation, hatching failures or brood reduction, and in another 12, they fledged exclusively shiny cowbirds. Overall, 106 nests that reached fledgling stage met our criteria (see Materials and Methods) and were included in the analysis of nestling survival.

During model selection of nestling survival analysis, the interaction between number of helpers and presence of shiny cowbird nestling was included in all our better fitted models with its 95% confidence interval excluding 0 (Tables 1 and 2). Therefore, we analysed the trend effect of helpers 'a posteriori' using the package emmeans (Lenth et al., 2020). The effect of helpers depended on the presence of shiny cowbird (z ratio $= 3.413$, $p = 0.0006$). In nests without shiny cowbird nestlings, number of helpers increased nestling survival (95% CI of slope β (0.826, 3.095)) (Figure 1). However, they did not have any effect when a shiny cowbird nestling was in the nest (95% CI of β (-0.986, 0.354)) (Figure 1). Nestling survival was unrelated to nest initiation date and total rainfall (Table 1).

3.2. Sex of helpers

From a total of 243 fledglings ringed during this study, 31 were re-sighted as helpers in subsequent breeding seasons. Of these, 28 (90.3%) were males and three (9.7%) were females. Observed proportion of male helpers was larger than 0.5 (95% CI 0.7425, 0.9796; exact binomial test, $p < 0.0001$) and larger than expected based on the sex of the 243 fledglings (see population
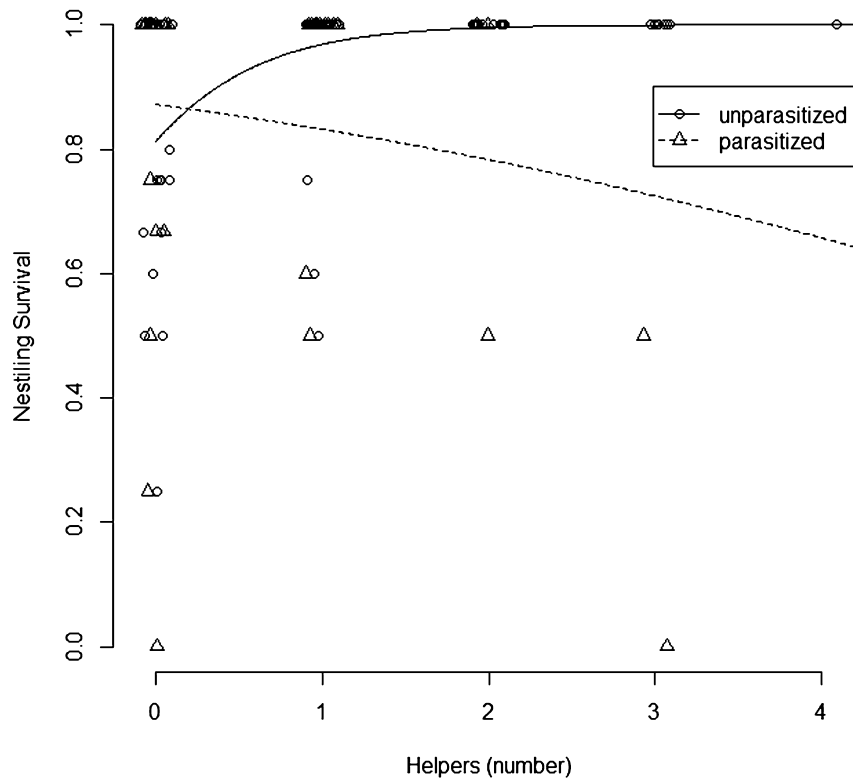
Figure 1. Effect of helpers on nestling survival. We show the probability of survival of brown-and-yellow marshbird nestlings relative to the number of helpers in broods without brood parasitism (unparasitized — dots) and in those in which a shiny cowbird (*Molothrus bonariensis*) nestling was present (parasitized — triangles). Symbols show observed values, lines values predicted by the model. Helpers increased nestling survival in unparasitized nests, but did not have any effect in parasitized ones.

Table 1.

Results of GLMM analysis of nestling survival in brown-and-yellow marshbird nests between 2001 and 2009: Summary of the multi-model selection approach.

Candidate model	K	AIC_c	ΔAIC_c	w_i
HELP × SC[1]	6	132.4	0.00	0.570
HELP × SC DATE	7	134.6	2.25	0.186
HELP × SC RAIN	7	134.6	2.28	0.182
HELP × SC DATE RAIN (global)	8	136.9	4.58	0.058
Null model	3	152.6	20.28	0.000

Models are ranked from highest to lowest relative likelihood (wi). Those with low support ($wi < 0.05$) were omitted, excepting the null model for comparison. K, number of parameters; AIC_c, Akaike's information criterion corrected for small sample sizes; ΔAIC_c, difference between AIC_c of the model specified and the lowest AIC_c.

[1] HELP × SC = HELP: SC + HELP + SC.

Table 2.

Results of GLMM analysis of nestling survival in brown-and-yellow marshbird nests between 2001 and 2009: Model-averaged parameter estimates (±SE) and 95% confidence intervals (CI) for the explanatory variables.

Explanatory variable	Parameter (estimate ± SE)	CL (95%)	
		Lower	Upper
Intercept	*1.435 ± 0.682*	*0.081*	*2.788*
HELP	*1.962 ± 0.577*	*0.817*	*3.107*
SC (yes)	0.447 ± 0.555	−0.654	1.549
HELP:SC	*−2.280 ± 0.666*	*−3.601*	*−0.959*
RAIN	0.0005 ± 0.0005	−0.012	0.011
DATE	0.000 ± 0.015	−0.012	0.011

Explanatory variables were number of helpers (HELP), presence of shiny cowbird nestlings (SC), interaction between number of helpers and presence of shiny cowbird nestlings (HELP:SC), summed rainfall during the nestling stage and the 15 previous days (RAIN), and Julian date of nest initiation (DATE). In all models, group identity and year were added as random factors. CI excluding zero are in italics.

brood sex-ratio, exact binomial test $p = 0.0002$ for a proportion of 0.59 males). Most individuals (15 males and one female) helped their parents and nine (seven males and two females) were helping at nests of non-parent individuals located close to their natal nesting site. For the remaining six males, the identity of the nest owners could not be determined. Except in one case where parents were simultaneously breeding, instances of helping non-parent individuals coincided with parent's nest failures early in the breeding cycle or parents breeding late or not breeding at all in that breeding season. Therefore, helping their parents appear to be the first option for helpers. Helpers were usually re-sighted with or near their natal group and apparently did not try to breed independently before helping. The oldest male helper remained with his parents and helped until he was 3 years-old. When his father died, this helper inherited the territory and could breed independently. The only female who helped her parents did so after losing her own nest due to predation during same breeding season.

3.3. Brood sex-ratio

We sexed 243 fledglings from 93 broods initially containing 410 eggs. Sixteen broods were complete at the end of the nestling stage (day 7), the remaining 77 broods lost 167 eggs or nestlings. One hundred and four eggs

Table 3.

Results of GLMM analysis of brood sex-ratio in brown-and-yellow marshbirds between 2001–2011: Summary of the multi-model selection approach.

Candidate model	K	AIC_c	ΔAIC_c	w_i
BL DATE	5	219.0	0.00	0.273
BL DATE HELP	6	219.8	0.80	0.182
BL	4	220.9	1.97	0.102
BL DATE RAIN	6	221.0	1.99	0.101
BL DATE HELP RAIN (global)	7	221.6	2.65	0.073
Null model	3	223.9	4.93	0.023

Models are ranked from highest to lowest relative likelihood (wi). Those with low support ($wi < 0.05$) were omitted, excepting the null model for comparison. K, number of parameters; AIC_c, Akaike's information criterion corrected for small sample sizes; ΔAIC_c, difference between AIC_c of the model specified and the lowest AIC_c.

were lost due to hatching failures, 46 eggs or nestlings due to partial depredation, and 17 nestlings due to brood reduction.

At the population level, brood sex-ratio (BSR) was male-biased, with 144 male and 99 female fledglings, respectively (sex-ratio $= 0.593$; 95% CI $= [0.528–0.655]$; exact binomial probability: $p = 0.0008$). At individual level, brood sex-ratio was affected by brood losses and nest initiation date (Table 3). Proportion of males increased with brood losses (Table 4, Figure 2). In addition, sex-ratio was less male-biased as the breeding season advanced (Table 4, Figure 3). BSR did not depend on the number of helpers, or total rainfall (Tables 3 and 4).

4. Discussion

The repayment hypothesis states that if helpers are mainly of one sex and provide benefits to the reproductive success of parental individuals, breeding females will bias the sex-ratio of their broods towards the helping sex in an inversely proportional way to the number of helpers assisting the nest. We tested the premises and predictions of the repayment hypothesis in a brown-and-yellow marshbird population and found that the presence of helpers increased nestling survival and that helping individuals were mostly males. We also found that fledgling sex-ratio was biased towards males, independently of the number of helpers assisting the nest.

Table 4.

Results of GLMM analysis of brood sex-ratio in brown-and-yellow marshbirds between 2001–2011: Model-averaged parameter estimates (±SE) and 95% confidence intervals (CI) for the explanatory variables.

Explanatory variable	Parameter (estimate ± SE)	CL (95%)	
		Lower	Upper
Intercept	0.946 ± 0.803	-0.623	2.520
BL	*1.651 ± 0.722*	*0.235*	*3.068*
DATE	*-0.019 ± 0.009*	*-0.038*	*-0.001*
HELP	-0.212 ± 0.185	-0.584	0.144
RAIN	-0.002 ± 0.037	-0.009	0.005

Explanatory variables were number of helpers (HELP), brood losses (BL), Julian date of nest initiation (DATE), and summed rainfall during egg formation and the 15 previous days (RAIN). In all models, female identity and year were added as random factors. CI excluding zero are in italics.
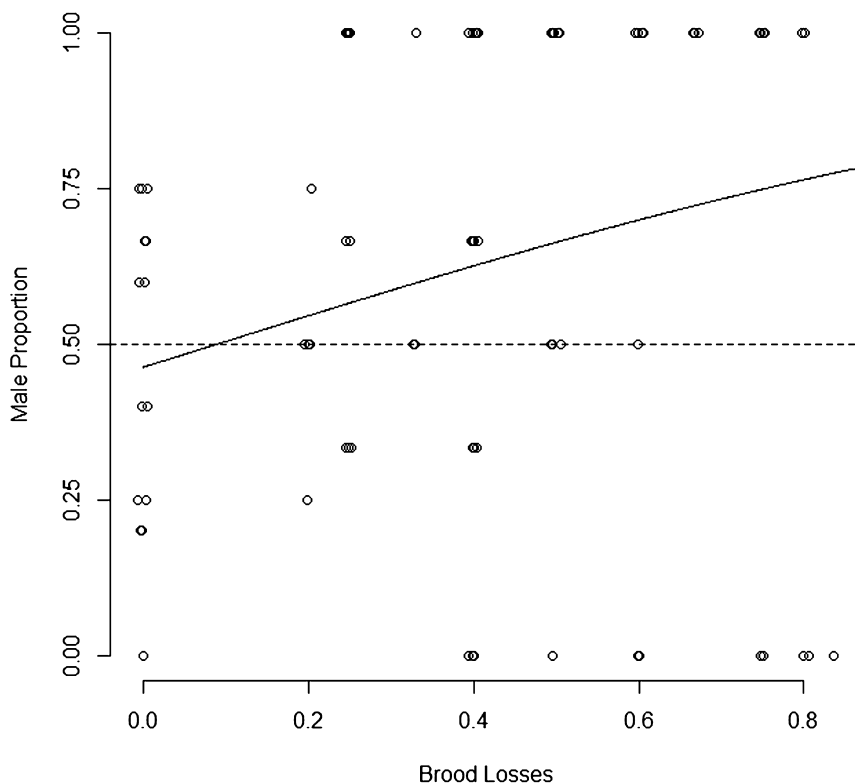
Figure 2. Effect of brood completeness on brood sex-ratio. We show the proportion of males in broods of brown-and-yellow marshbirds relative to brood losses. Brood losses refer to the proportion of the original brood that was lost due to partial predation of egg or nestlings, hatching failures or brood reduction. Symbols show observed values, the solid line values predicted by the model. The horizontal dashed line indicates equal proportion between males and females.
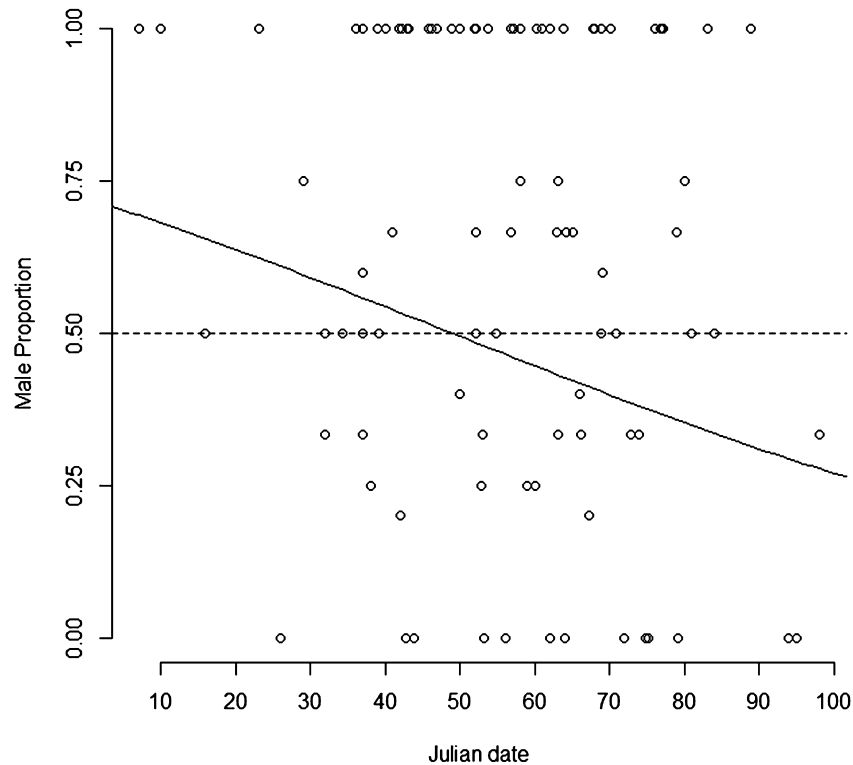
Figure 3. Effect of date along the breeding season on brood sex-ratio. We show the proportion of males in broods of brown-and-yellow marshbirds relative to nest initiation date (Julian date 1 = 1 September). Symbols show observed values, the solid line values predicted by the model. The horizontal dashed line indicates equal proportion between males and females.

4.1. Effect of helpers on nestling survival

In brown-and-yellow marshbirds, nests attended by more helpers had an increase in nestlings' survival. This is in accordance with the video recordings showing that helpers increased overall food provisioning to the nest, measured as number and size of prey items delivered, resulting in higher *per capita* feeding (Mermoz et al., 2008; Mermoz, 2011). However, this effect disappeared when a shiny cowbird chick was in the nest. In nests of smaller hosts, shiny cowbird chicks monopolize a disproportionally large portion of deliveries due to their larger size (Tuero et al., 2016; Bortolato et al., 2019). Although marshbird nestlings are larger than shiny cowbirds, the latter hatch 1–2 days ahead due to the good synchronization of parasitism with host egg-laying and their shorter incubation period (Mermoz & Reboreda, 1999, 2003). As shiny cowbird chicks also have a more accelerated growing rate, they are the largest nestlings in marshbird nests (Mermoz & Reboreda, 2003). Therefore, shiny cowbirds might monopolize most deliveries of food items, neutralizing the positive effect on nestling survival that helpers cause

in unparasitized nests. In addition, helpers defend the nest against predators including cowbirds (Orians, 1980; Mermoz et al., 2013). Consequently, nests defended by larger groups tend to receive fewer shiny cowbird eggs (M.E. Mermoz, pers. obs.), a pattern described for other cooperative breeders (Canestrari et al., 2009; Feeney et al., 2013). The summed effect of the larger size of shiny cowbird chicks and a smaller number of helpers may explain the lack of association between nestling survival and number of helpers in presence of shiny cowbird chicks in the nest.

4.2. Sex of helpers

Helpers at the nest were mainly males, with 9.33 males for every female. This bias towards males was even more pronounced than the one we detected in the broods at population level (see below). The prevalence of male helpers has been shown for a large number of cooperatively breeding birds (Ligon & Burt, 2004; Koenig et al., 2016; Komdeur et al., 2017). This pattern is probably related to female-biased dispersal behaviour in most avian species (Clarke et al., 1997; Mabry et al., 2013; Komdeur et al., 2017).

4.3. Brood sex-ratio

As expected under the repayment hypothesis, offspring sex-ratio was biased towards the helping sex (males) in this population of brown-and-yellow marshbirds. Brood sex-ratio might be skewed by parental sex-allocation strategies at the zygote state or sexual differences in embryo and nestling mortality (Svensson et al., 2007; Romano et al., 2012). In this study, we did not have information on primary sex-ratio and therefore did not directly test for these processes, but we found evidence that brood losses biased sex-ratio towards the helping sex. Within our study sample of 93 broods, only sixteen were complete, but those broods tended to have equal number of males and females. Sex-ratio became increasingly male-biased as the proportion of egg or nestling losses increased. Consequently, the estimated secondary sex-ratio at fledging was 1.4 males per female. At the egg stage, females might bias sex-ratio of their broods in favour of males by preferentially fertilizing or assigning resources to their male ova (Komdeur et al., 2002; Pike & Petrie, 2003; Young & Badyaev, 2004; Alonso-Álvarez, 2006; English et al., 2014). These mechanisms either would create infertile eggs (those carrying the female sex chromosome) or promote death of female embryo, and both might be in part responsible of the 25.4% egg losses due to hatching failure

of our original data set. Hatching failures are frequently reported in species with helpers at the nest (Spottiswoode & Møller, 2004). Other losses such as those caused by partial depredation of eggs or nestlings (11.2% of losses in our dataset) or death of nestlings by brood reduction (4.2% nestling losses) are, however, not in full control of the laying female. Once nestlings hatch, the attending male and helpers could also be manipulating brood sex-ratio by preferentially feeding male nestlings (Ridley & Huyvaert, 2007; Da et al., 2018). In addition, ecological conditions can differentially affect the survival of nestlings (Romano et al., 2012; Khwaja et al., 2017). Consequently, the male-biased secondary sex-ratio found in marshbirds might arise from the effects of any sex allocation strategy adopted by parents and helpers in conjunction with the effects of ecological conditions on survival of either sex (Sheldon et al., 1998; Ridley & Huyvaert, 2007; Khwaja et al., 2017; Da et al., 2018; Tschumi et al., 2019).

Contrary to predictions, we did not find that sex-ratio was more biased towards the helping sex in broods of females with few or no helpers, compared to females with helpers. Considering that parents might get help from individuals that are not close relatives, the number of helpers of the current reproductive attempt might not be a reliable predictor of the amount of help that breeders will get in future breeding attempts. Moreover, helpers might enhance parents' reproductive success besides increased nestling survival. Brown-and-yellow marshbirds usually produce one successful nest per breeding season. The only two cases of a second nesting attempt after a successfully fledged nest were observed in groups with 3–4 helpers who were left in charge of the fledglings of the first nest (M.E. Mermoz, unpub. data). Duplicating breeding success might also be an important incentive for brown-and-yellow marshbird's parents to produce more males.

Finally, we found that relatively early broods had a higher proportion of male nestlings than relatively late broods, consistent with previous studies on brood sex-ratios in raptors (Dijkstra et al., 1990; Daan et al., 1996; Smallwood & Smallwood, 1998; Griggio et al., 2002) and passerines (Andersson et al., 2003; Neto et al., 2011; Bowers et al., 2015). In most studies, the explanation for such bias relies on early males having higher survival or achieving faster and/or better opportunities for reproduction, known as the early bird hypothesis (Smallwood & Smallwood, 1998). In brown-and-yellow marshbirds, males do not acquire direct reproduction until they are at least three years old (M.E. Mermoz & G.J. Fernández, unpublished data).

Consequently, we consider that the early bird hypothesis would not apply to marshbirds. However, it is important to note that in passerines young that fledge earlier in the breeding season generally have a higher chance of surviving (Sheldon et al, 1998; Greño et al., 2008; Sim et al., 2013; Rodriguez et al., 2016).

Modifications to the original repayment model by Emlen et al. (1986) also consider the fitness benefits of producing the dispersing sex as individuals of that sex reproduce earlier (e.g. Koenig & Walters, 1999; Pen & Weissing, 2000). Even though the benefits of the dispersing sex are included, they still predict a population brood sex-ratio biased towards the helping sex when the benefits of helping outweigh fitness losses due to the increased competition when remaining with their parents (Koenig & Walters, 1999; Pen & Weissing, 2000; Wild, 2006). In brown-and-yellow marsbirds, helpers increased nestling survival and a large number of helpers even allowed duplicating breeding success of parents. Therefore. skewing brood sex-ratios towards the helping sex regardless of the number of helpers might be a more convenient strategy in this species.

4.4. *Evidence for the repayment hypothesis*

Since the repayment hypothesis was proposed (Emlen et al., 1986), a large number of studies on brood sex-ratio in species with helpers at the nest tested this hypothesis, obtaining dissimilar results (see the reviews of Cockburn et al., 2002; Komdeur, 2004, 2012; and the meta-analyses by Griffin et al., 2005; Khwaja et al., 2017 for a summary of results). Overall, species conforming to the repayment hypothesis were the exception rather than the rule (Khwaja et al., 2017). In general, different outcomes will be expected with varying strengths of selective pressures related to the benefits obtained from the helping sex (Griffin et al., 2005) and other factors that influence offspring sex allocation, like unpredictable environments, unpredictable help, and social structure (Khwaja et al., 2017). The two species that more clearly meet the predictions of the hypothesis are the bell miner (*Manorina melanophrys*) and the Seychelles warbler (*Acrocephalus sechellensis*). When food is not limited, both species bias sex-ratio toward the helping sex at population level (Clarke et al., 2002; Komdeur, 2003). At individual level, females bias sex-ratio toward helpers or the dispersing sex depending more on food availability than on the presence of helpers (Ewen et al., 2003; Komdeur, 2003; 2004). Like in these species, food is not limiting in brown-and-yellow marshbirds as they do not defend feeding areas and forage in

flocks with other members of the population (Orians, 1980). Retaining more helpers would not be costly and therefore, females can bias sex-ratio of their broods towards males independently of the number of helpers assisting the nest. However, the correlational nature of our data does not allow us to determine the chain of causation of the observed pattern and a prevalence of male helpers might arise from a biased sex-ratio that leaves more males unpaired and thus forced to help (Komdeur et al., 2017). Regardless of its origin, this initial excess of males might have important implications for the evolution and maintenance of cooperative breeding in brown-and-yellow marshbirds.

To summarise, we found that fledglings are skewed towards the helping sex in a resident population of a cooperatively breeding passerine with facultative helping that does not defend feeding areas. Helpers were mostly males and contributed to the reproductive outcome of parental individuals, which were not always their parents. Therefore, a general skew in sex-ratio towards males may be adaptive and might explain the absence of an association between sex-ratio bias and the help females are getting during their ongoing reproductive attempt.

Acknowledgements

References

Alonso-Álvarez, C. (2006). Manipulation of primary sex ratio: an updated review. — Avian Poult. Biol. Rev. 17: 1-20.

Andersson, M., Wallander, J., Oring, L., Akst, E., Reed, J.M. & Fleischer, R.C. (2003). Adaptive seasonal trend in brood sex ratio: test in two sister species with contrasting breeding systems. — J. Evol. Biol. 16: 510-515.

Barnett, K.L. & Facey, S.L. (2016). Grasslands, invertebrates, and precipitation: a review of the effects of climate change. — Front. Plant Sci. 7: 1196.

Benito, M.M. & González-Solís, J. (2007). Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds. — J. Evol. Biol. 20: 1522-1530.

Berg, E.C. (2004). A test of sex-ratio biasing in the white-throated magpie-jay, a cooperative breeder with female helpers. — Condor 106: 299-308.

Bortolato, T., Gloag, R., Reboreda, J.C. & Fiorini, V.D. (2019). Size matters: shiny cowbirds secure more food than host nestmates thanks to their larger size, not signal exaggeration. — Anim. Behav 157: 201-207.

Bowers, E.K., Thompson, C.F. & Sakaluk, S.K. (2015). Persistent sex-by-environment effects on offspring fitness and sex-ratio adjustment in a wild bird population. — J. Anim. Ecol. 84: 473-486.

Brown, J.L. (1987). Helping and communal breeding in birds: ecology and evolution. — Princeton University Press, Princeton, NJ.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: a practical information-theoretic approach. — Springer, New York, NY.

Canestrari, D., Marcos, J.M. & Baglione, V. (2009). Cooperative breeding in carrion crows reduces the rate of brood parasitism by great spotted cuckoos. — Anim. Behav. 77: 1337-1344.

Canestrari, D., Vila, M., Marcos, J.M. & Baglione, V. (2012). Cooperatively breeding carrion crows adjust offspring sex ratio according to group composition. — Behav. Ecol. Sociobiol. 66: 1225-1235.

Clarke, A.L., Sæther, B.E. & Røskaft, E. (1997). Sex biases in avian dispersal: a reappraisal. — Oikos 79: 429-438.

Clarke, M.F., Jones, D.A.G.E.J., Robertson, R.J., Griffiths, R., Painter, J., Boag, P.T. & Crozier, R. (2002). Male-biased sex ratios in broods of the cooperatively breeding bell miner *Manorina melanophrys*. — J. Avian Biol. 33: 71-76.

Clout, M.N., Elliott, G.P. & Robertson, B.C. (2002). Effects of supplementary feeding on the offspring sex ratio of kakapo: a dilemma for the conservation of a polygynous parrot. — Biol. Conserv. 107: 13-18.

Clutton-Brock, T. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. — Science 296: 69-72.

Cockburn, A. (1998). Evolution of helping behavior in cooperative breeding birds. — Annu. Rev. Ecol. Syst. 29: 141-177.

Cockburn, A. & Double, M.C. (2008). Cooperatively breeding superb fairy-wrens show no facultative manipulation of offspring sex ratio despite plausible benefits. — Behav. Ecol. Sociobiol. 62: 681-688.

Cockburn, A. & Russel, A.F. (2011). Cooperative breeding: a question of climate?. — Curr. Biol. 21: 195-197.

Cockburn, A., Legge, S. & Double, M.C. (2002). Sex ratios in birds and mammals: can the hypotheses be disentangled. — In: Sex ratios: concepts and research methods (Hardy, I.C., ed.). Cambridge University Press, Cambridge, p. 266-286.

Covas, R., Du Plessis, M.A. & Doutrelant, C. (2008). Helpers in colonial cooperatively breeding sociable weavers *Philetairus socius* contribute to buffer the effects of adverse breeding conditions. — Behav. Ecol. Sociobiol. 63: 103-112.

Crawley, M.J. (2007). The R book. — Wiley, Chichester.

Da, X.W., Xian, L.L., Luo, J.J. & Du, B. (2018). Parents of the azure-winged magpie exhibit sex-specific allocation of food to offspring. — J. Ornithol. 159: 255-263.

Daan, S., Dijkstra, C. & Weissing, F.J. (1996). An evolutionary explanation for seasonal trends in avian sex ratios. — Behav. Ecol. 7: 426-430.

Dijkstra, C., Daans, S. & Buker, J.B. (1990). Adaptive seasonal variation in the sex ratio of kestrel broods. — Funct. Ecol. 4: 143-147.

Doutrelant, C., Covas, R., Caizergues, A. & Du Plessis, M. (2004). Unexpected sex ratio adjustment in a colonial cooperative bird: pairs with helpers produce more of the helping sex whereas pairs without helpers do not. — Behav. Ecol. Sociobiol. 56: 149-154.

Duré Ruiz, N.M., Mermoz, M.E. & Fernández, G.J. (2008). Effect of cowbird parasitism on brood reduction in the brown-and-yellow marshbird. — Condor 110: 507-513.

Dyrcz, A., Saber-Gürth, H., Tkadlec, E. & Wink, M. (2004). Offspring sex ratio variation in relation to brood size and mortality in a promiscuous species: the aquatic warbler *Acrocephalus paludicola*. — Ibis 146: 269-280.

Emlen, S.T., Emlen, J.M. & Levin, S.A. (1986). Sex-ratio selection in species with helpers-at-the-nest. — Am. Nat. 127: 1-8.

English, W.D., Schamel, D., Tracy, D.M., Westneat, D.F. & Lank, D.B. (2014). Sex ratio varies with egg investment in the red-necked phalarope (*Phalaropus lobatus*). — Behav. Ecol. Sociobiol. 68: 1939-1949.

Ewen, J.G., Crozier, R.H., Cassey, P., Ward-Smith, T., Painter, J.N., Robertson, R.J., Jones, D.A. & Clarke, M.F. (2003). Facultative control of offspring sex in the cooperatively breeding bell miner, *Manorina melanophrys*. — Behav. Ecol. 14: 157-164.

Feeney, W.E., Medina, I., Somveille, M., Heinsohn, R., Hall, M.L., Mulder, R.A., Stein, J.A., Kilner, R.M. & Langmore, N.E. (2013). Brood parasitism and the evolution of cooperative breeding in birds. — Science 342: 1506-1508.

Fiala, K.L. (1980). On estimating the primary sex ratio from incomplete data. — Am. Nat. 115: 442-444.

Fisher, R.A. (1930). The genetical theory of natural selection. — Oxford University Press, Oxford.

Greenwood, P.J. (1980). Mating systems, philopatry and dispersal in birds and mammals. — Anim. Behav. 28: 1140-1162.

Greño, J.L., Belda, E.J. & Barba, E. (2008). Influence of temperatures during the nestling period on post-fledging survival of great tit *Parus major* in a Mediterranean habitat. — J. Avian Biol. 39: 41-49.

Griesser, M., Drobniak, S.M., Nakagawa, S. & Botero, C.A. (2017). Family living sets the stage for cooperative breeding and ecological resilience in birds. — PLoS Biol. 15: e2000483. DOI:10.1371/journal.pbio.2000483.

Griffin, A.S., Sheldon, B.C. & West, S.A. (2005). Cooperative breeders adjust offspring sex ratios to produce helpful helpers. — Am. Nat. 166: 628-632.

Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J. (1998). A DNA test to sex most birds. — Mol. Ecol. 7: 1071-1075.

Griggio, M., Hamerstrom, G., Rosenfield, RN. & Tavecchia, G. (2002). Seasonal variation in sex ratio of fledgling American kestrels: a long term study. — Wilson Bull. 114: 474-478.

Hamilton, W.D. (1967). Extraordinary sex ratios. — Science 156: 477-488.

Hartig, F. (2020). Package 'DHARMa': residual diagnostics for hierarchical (multi-level/mixed) regression models. Available online at http://florianhartig.github.io/DHARMa/.

Hervé, M. (2020). Package 'RVAideMemoire'. Available online at https://CRAN.R-project.org/package=RVAideMemoire.

Hidalgo Aranzamendi, N., Hall, M.L., Kingma, S.A., van de Pol, M. & Peters, A. (2019). Rapid plastic breeding response to rain matches peak prey abundance in a tropical savanna bird. — J. Anim. Ecol. 88: 1799-1811.

Jetz, W. & Rubenstein, D.R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. — Curr. Biol. 21: 72-78.

Juola, F.A. & Dearborn, D.C. (2007). Does the differential cost of sons and daughters lead to sex ratio adjustment in great frigate birds *Fregata minor*?. — J. Avian Biol. 38: 94-104.

Keynan, O. & Ridley, A.R. (2016). Component, group and demographic Allee effects in a cooperatively breeding bird species, the Arabian babbler (*Turdoides squamiceps*). — Oecologia 182: 153-161.

Khwaja, N., Hatchwell, B.J., Freckleton, R.P. & Green, J.P. (2017). Sex allocation patterns across cooperatively breeding birds do not support predictions of the repayment hypothesis. — Am. Nat. 190: 547-556.

Khwaja, N., Preston, S.A., Briskie, J.V. & Hatchwell, B.J. (2018). Testing the predictions of sex allocation hypotheses in dimorphic, cooperatively breeding riflemen. — Ecol. Evol. 8: 3693-3701.

Kingma, S.A., Hall, M.L. & Peters, A. (2011). No evidence for offspring sex-ratio adjustment to social or environmental conditions in cooperatively breeding purple-crowned fairy-wrens. — Behav. Ecol. Sociobiol. 65: 1203-1213.

Koenig, W.D. (2017). What drives cooperative breeding? — PLoS Biol. 15: e2002965.

Koenig, W.D. & Dickinson, J.L. (1996). Nestling sex-ratio variation in western bluebirds. — Auk 113: 902-910.

Koenig, W.D. & Walters, J.R. (1999). Sex-ratio selection in species with helpers at the nest: the repayment model revisited. — Am. Nat 153: 124-130.

Koenig, W.D., Walters, E.L. & Haydock, J. (2011). Variable helper effects, ecological conditions, and the evolution of cooperative breeding in the acorn woodpecker. — Am. Nat 178: 145-158.

Koenig, W.D., Dickinson, J.L. & Emlen, S.T. (2016). Synthesis: cooperative breeding in the twenty-first century. — In: Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior (Koenig, W.D. & Dickinson, J.L., eds). Cambridge University Press, Cambridge, p. 353-374.

Komdeur, J. (1996). Facultative sex ratio bias in the offspring of Seychelles warblers. — Proc. Roy. Soc. Lond. B: Biol. Sci. 263: 661-666.

Komdeur, J. (2003). Daughters on request: about helpers and egg sexes in the Seychelles warbler. — Proc. Roy. Soc. Lond. B: Biol. Sci. 270: 3-11.

Komdeur, J. (2004). Sex-ratio manipulation. — In: Ecology and evolution of cooperative breeding in birds (Koenig, W.D. & Dickinson, J.L., eds). Cambridge University Press, Cambridge, p. 102-116.

Komdeur, J. (2012). Sex allocation. — In: The evolution of parental care (Royle, N.J., Smiseth, P.T. & Kölliker, M., eds). Oxford University Press, Oxford, p. 171-188.

Komdeur, J., Daan, S., Tinbergen, J. & Mateman, C. (1997). Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. — Nature 385: 522-525.

Komdeur, J., Magrath, M.J. & Krackow, S. (2002). Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. — Proc. Roy. Soc. Lond. B: Biol. Sci. 269: 1067-1072.

Komdeur, J., Burke, T., Dugdale, H. & Richardson, D.S. (2016). Seychelles warblers: complexities of the helping paradox. — In: Cooperative breeding in vertebrates: studies of ecology, evolution, and behaviour (Koenig, W.D. & Dickinson, J.L., eds). Cambridge University Press, Cambridge, p. 197-216.

Komdeur, J., Székely, T., Long, X. & Kingma, S.A. (2017). Adult sex ratios and their implications for cooperative breeding in birds. — Phil. Trans. Roy. Soc. Lond. B: Biol. Sci. 372: 20160322.

Krackow, S. & Neuhäuser, M. (2008). Insight from complete-incomplete brood sex-ratio disparity. — Behav. Ecol. Sociobiol. 62: 468-477.

Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. (2020). emmeans: estimated marginal means. R package version 1.4. 4.

Ligon, J.D. & Burt, D.B. (2004). Evolutionary origins. — In: Ecology and evolution of cooperative breeding in birds (Koenig, W.D. & Dickinson, J.L., eds). Cambridge University Press, Cambridge, p. 5-34.

Lukas, D. & Clutton-Brock, T. (2012). Cooperative breeding and monogamy in mammalian societies. — Proc. Roy. Soc. Lond. B: Biol. Sci. 279: 2151-2156.

Mabry, K.E., Shelley, E.L., Davis, K.E., Blumstein, D.T. & Van Vuren, D.H. (2013). Social mating system and sex-biased dispersal in mammals and birds: a phylogenetic analysis. — PLoS ONE 8: e57980.

McNew, S.M., Knutie, S.A. & Clayton, D.H. (2020). No evidence of sex ratio manipulation by Galápagos mockingbirds in response to environment. — J. Avian Biol.: 51. DOI:10. 1111/jav.02302.

Mermoz, M.E. (2011). Inversión parental en el pecho amarillo (*Pseudoleistes virescens*), una especie con cría cooperativa. — XIV Reunión Argentina de Ornitología, Formosa, Argentina.

Mermoz, M.E. & Reboreda, J.C. (1998). Nesting success in brown-and-yellow marshbirds: effects of timing, nest site and brood parasitism. — Auk 115: 871-878.

Mermoz, M.E. & Reboreda, J.C. (1999). Egg-laying behaviour by shiny cowbirds parasitizing brown-and-yellow marshbirds. — Anim. Behav. 58: 873-882.

Mermoz, M.E. & Reboreda, J.C. (2003). Breeding success of shiny cowbirds parasitizing the larger brown-and-yellow marshbird in Argentina. — Auk 120: 1128-1139.

Mermoz, M.E., Fernández, G.J. & Mahler, B. (2008). Helping behavior in the brown-and-yellow marshbird (*Pseudoleistes virescens*): analyzing the advantages for females. — XII International Behavioral Ecology Congress, Ithaca, NY.

Mermoz, M.E., Reboreda, J.C. & Fernández, G.J. (2013). High rates of brood parasitism by shiny cowbird on brown-and-yellow marshbirds select for complementary host defenses. — Condor 115: 919-920.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. — Nucleic Acids Res. 16: 1215.

Moreno-Rueda, G., Campos, F., Gutiérrez-Corchero, F. & Hernández, M.Á. (2014). Costs of rearing and sex-ratio variation in southern grey shrike *Lanius meridionalis* broods. — J. Avian Biol. 45: 424-430.

Nam, K.B., Meade, J. & Hatchwell, B.J. (2011). Brood sex ratio variation in a cooperatively breeding bird. — J. Evol. Biol. 24: 904-913.

Napper, C.J. & Hatchwell, B.J. (2016). Social dynamics in nonbreeding flocks of a cooperatively breeding bird: causes and consequences of kin associations. — Anim. Behav. 122: 23-35.

Nelson-Flower, M.J., Flower, T.P. & Ridley, A.R. (2018). Sex differences in the drivers of reproductive skew in a cooperative breeder. — Mol. Ecol. 27: 2435-2446.

Neto, J.M., Hansson, B. & Hasselquist, D. (2011). Sex allocation in Savi's warblers *Locustella luscinioides*: multiple factors affect seasonal trends in brood sex ratios. — Behav. Ecol. Sociobiol. 65: 297-304.

Orians, G.H. (1980). Some adaptations of Marsh-nesting blackbirds. — Princeton University Press, Princeton, NJ.

Pen, I. & Weissing, F.J. (2000). Sex-ratio optimization with helpers at the nest. — Proc. R. Soc. Lond. B: Biol. Sci. 267: 539-543.

Perrins, C.M. (1994). Eggs, eggs formation and timing of breeding. — Ibis 138: 2-15.

Pike, T.W. & Petri, M. (2003). Potential mechanisms of avian sex manipulation. — Biol. Rev. 78: 553-574.

R Development Core Team (2019). R: a language and environment for statistical computing. — R Foundation for Statistical Computing, Vienna. Available online at http://www.r-project.org/.

Ribeiro, B.A., Carrara, L.A., Santos, F.R. & Rodrigues, M. (2007). Sex-biased help and possible facultative control over offspring sex ratio in the rufous-fronted thornbird *Phacellodomus rufifrons*. — Rev. Bras. Ornitol. 15: 561-568.

Ridley, A.R. (2016). Southern pied babblers: the dynamics of conflict and cooperation in a group-living society. — In: Cooperative breeding in vertebrates: studies of ecology,

evolution, and behaviour (Koenig, W.D. & Dickinson, J.L., eds). Cambridge University Press, Cambridge, p. 115-132.

Ridley, A.R. & Huyvaert, K.P. (2007). Sex-biased preferential care in the cooperatively breeding Arabian babbler. — J. Evol. Biol. 20: 1271-1276.

Rodrıguez, S., Van Noordwijk, A.J., Álvarez, E. & Barba, E. (2016). A recipe for postfledging survival in great tits *Parus major*: be large and be early (but not too much). — Ecol. Evol. 6: 4458-4467.

Romano, A., Ambrosini, R., Caprioli, M., Bonisoli-Alquati, A. & Saino, N. (2012). Secondary sex ratio covaries with demographic trends and ecological conditions in the barn swallow. — Evol. Ecol. 26: 1041-1053.

Rubenstein, D.R. (2007). Temporal but not spatial environmental variation drives adaptive offspring sex allocation in a plural cooperative breeder. — Am. Nat. 170: 155-165.

Rubenstein, D.R. & Lovette, I.J. (2007). Temporal environmental variability drives the evolution of cooperative breeding in birds. — Curr. Biol. 17: 1414-1419.

Rutkowska, J. & Badyaev, A.V. (2008). Review, meiotic drive, and sex determination: molecular and cytological mechanism of sex ratio adjustment in birds. — Phil. Trans. Roy. Soc. Lond. B: Biol. Sci. 1497: 1675-1686.

Seward, A.M., Beale, C.M., Gilbert, L., Jones, T.H. & Thomas, R.J. (2013). The impact of increased food availability on survival of a long-distance migratory bird. — Ecology 94: 221-230.

Sheldon, B.C., Merilä, J., Lindgren, G. & Ellegren, H. (1998). Gender and environmental sensitivity in nestling collared flycatchers. — Ecology 79: 1939-1948.

Shen, S.F., Emlen, S.T., Koenig, W.D. & Rubenstein, D.R. (2017). The ecology of cooperative breeding behaviour. — Ecol. Lett. 20: 708-720.

Sim, I.M., Ludwig, S.C., Grant, M.C., Loughrey, J.L., Rebecca, G.W. & Reid, J.M. (2013). Postfledging survival, movements, and dispersal of ring ouzels (*Turdus torquatus*). — Auk 130: 69-77.

Smallwood, P.D. & Smallwood, J.A. (1998). Seasonal shifts in sex ratios of fledgling American kestrels (*Falco sparverius paulus*): the early bird hypothesis. — Evol. Ecol. 12: 839-853.

Sorato, E., Griffith, S.C. & Russell, A.F. (2016). The price of associating with breeders in the cooperatively breeding chestnut-crowned babbler: foraging constraints, survival and sociality. — J. Anim. Ecol. 85: 1340-1351.

Soriano, A., León, R.J.C., Sala, O.E., Lavado, R.S., Deregibus, V.A., Cauhepé, M.A., Scaglia, O.A., Velázquez, C.A. & Lemcoff, J.H. (1992). Río de la Plata Grasslands. — In: Ecosystems of the world: natural grasslands. Introduction and western hemisphere (Coupland, R.T., ed.). Elsevier, Amsterdam, p. 367-407.

Spottiswoode, C.N. & Møller, A.P. (2004). Genetic similarity and hatching success in birds. — Proc. Roy. Soc. Lond. B: Biol. Sci. 271: 267-272.

Svensson, M., Rintamäki, P.T., Birkhead, T.R., Griffith, S.C. & Lundberg, A. (2007). Impaired hatching success and male-biased embryo mortality in Tree Sparrows. — J. Ornithol. 148: 117-122.

Szczys, P., Nisbet, I.C., Hatch, J.J. & Kesseli, R.V. (2001). Sex ratio bias at hatching and fledging in the roseate tern. — Condor 103: 385-389.

Trivers, R.L. & Willard, D. (1973). Natural selection of parental ability to vary the sex ratio of offspring. — Science 179: 90-92.

Trnka, A., Prokop, P., Kašová, M., Sobeková, K. & Kocian, L. (2012). Hatchling sex ratio and female mating status in the great reed warbler, *Acrocephalus arundinaceus* (Aves, Passeriformes): further evidence for offspring sex ratio manipulation. — Ital. J. Zool. 79: 212-217.

Tschumi, M., Humbel, J., Erbes, J., Fattebert, J., Fischer, J., Fritz, G., Geiger, B., van Harxen, R., Hoos, B., Hurst, J., Jacobsen, L.B., Keil, H., Kneule, W., Michel, V.T., Michels, H., Möbius, L., Perrig, M., Rößler, P., Schneider, D., Schuch, S., Stroeken, P., Naef-Daenzer, B. & Grüebler, M.U. (2019). Parental sex allocation and sex-specific survival drive offspring sex ratio bias in little owls. — Behav. Ecol. Sociobiol. 73: 85.

Tuero, D.T., Gloag, R. & Reboreda, J.C. (2016). Nest environment modulates begging behavior of a generalist brood parasite. — Behav. Ecol. 27: 204-210.

Verboven, N., Tinbergen, J.M. & Verhulst, S. (2001). Food, reproductive success and multiple breeding in the great tit *Parus major*. — Ardea 89: 387-406.

Wiehn, J. & Korpimäki, E. (1997). Food limitation on brood size: experimental evidence in the Eurasian kestrel. — Ecology 78: 2043-2050.

Wild, G. (2006). Sex ratios when helpers stay at the nest. — Evolution 60: 2012-2022.

Williams, G.C. (1979). The question of adaptive sex ratio in outcrossed vertebrates. — Proc. R. Soc. Lond. B Biol. 205: 567-580.

Woxvold, I.A. & Magrath, M.J. (2008). Sex-biases in the hatching sequence of cooperatively breeding apostlebirds *Struthidea cinerea*. — Evol. Ecol. 22: 139-151.

Wright, J. & Russell, A.F. (2008). How helpers help: disentangling ecological confounds from the benefits of cooperative breeding. — J. Anim. Ecol. 77: 427-429.

Young, R.L. & Badyaev, A.V. (2004). Evolution of sex-biased maternal effects in birds: I. Sex-specific resource allocation among simultaneously growing oocytes. — J. Evol. Biol. 17: 1355-1366.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). Mixed effects models and extensions in ecology with R. — Springer Science & Business Media, New York, NY.