

The role of the mating system and intraspecific brood parasitism in the costs of reproduction in a passerine bird

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Abstract Limited resources trigger trade-offs in resource allocation. Reproduction is one of the most demanding activities in terms of energy, and costs related to reproduction can be apparent either as reduced future reproduction or as survival. However, costs are not always detected, because individual heterogeneity in quality and resource acquisition may explain variation in costs among individuals. We conducted a comprehensive study in spotless starlings (*Sturnus unicolor*) nesting in Central Spain to determine whether costs of reproduction could be detectable in the short and/or long term and whether prenatal or postnatal investment were driving these costs. We took into account the life history of the species, where egg volume is highly repeatable, males are facultatively polygynous, and intraspecific brood parasitism occurs. Females with a high reproductive effort in a breeding event also had a high reproductive effort in the second event of the season. The mating system and maternal age were reliable predictors of breeding success within

a breeding event: monogamous and primary females, as well as older mothers, raised more nestlings than secondary females and 1-year-old females, respectively. However, when high-investing females suffered intra-brood parasitism in 1 year, we found a negative correlation between current and future reproduction. These results suggest that, under some circumstances, females are able to skip the short-term costs of reproduction, but when extra effort is added, trade-offs arise. While most studies have focused on different aspects of individual quality on costs, the ecological context seems to represent an important component that should be taken into account.

Keywords Egg volume · Prenatal investment · Postnatal investment · Reproduction · Spotless starling · *Sturnus unicolor*

Introduction

The theory of life-history trade-offs is based on the notion that an organism can only acquire a finite amount of resources to replenish energetic demands (Roff 1992; Stearns 1992). Reproduction is one of the most energetically demanding activities, and it is assumed that current reproduction compromises either future reproduction and/or survival (Clutton-Brock et al. 1983).

Different reproductive tactics have evolved to deal with the costs of reproduction, with consequences at the individual and the population level (Proaktor et al. 2008). However, at the individual level, costs are not always detected (Hamel et al. 2010; Moyes et al. 2011; Santos and Nakagawa 2012). Costs might be state and context-dependent (Barbraud and Weimerskirch 2005; Beauplet et al. 2006; Cam et al. 2002). For instance, individual animals differ in their

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resource acquisition and allocation abilities (van Noordwijk and de Jong 1986). Thus, females that are better able to exploit resources might reduce the purported cost of reproduction (Rödel et al. 2016). In iteroparous species, skills related to resource monopolization are honed through life, and therefore, the costs of reproduction vary with age (Cam et al. 2002; Hegyi et al. 2006). The ecological context has a strong influence on these processes. For instance, the breeding system is an important modulator of reproductive effort in a given breeding season, particularly in species with biparental care. Females mating with polygynous males might receive less male assistance (Sandell et al. 1996; Slagsvold and Lifjeld 1994), and even in monogamous systems, mismatches between pair members are commonplace; typically, females compensate for reduced male parental investment (Sanz et al. 2000), which might perturb the costs for each of the parties.

Despite having evolved several mechanisms to cope with reproductive costs, animals nevertheless face constraints. A good example comes from birds, where egg volume of individual females is more or less constant through their life (Christians 2002), and therefore, a given amount of prenatal investment is set constant from the beginning. This prenatal investment should also correlate with higher incubation and rearing costs. For instance, it has been shown that incubation costs are non-negligible (de Heij et al. 2006; Leach et al. 2017; Monaghan and Nager 1997; Sanz 1997), and females laying larger eggs might incur higher prenatal costs than females laying smaller eggs because of the higher allocation of resources to egg production (Slattery and Alisauskas 1995). Moreover, these higher prenatal costs might translate into higher postnatal costs, as nestlings hatching from larger eggs are bigger, and have higher energetic demands, at least during the early post-hatching period (Slattery and Alisauskas 1995; Smith et al. 1995). Clutch size is usually more plastic than egg volume (Christians 2002), and thus, females could modulate investment by adjusting the number of eggs laid. However, the trade-off between egg volume and number is not apparent when resources and acquisition abilities vary, as females in good body condition might be able to both lay large clutches with large eggs (Kvalnes et al. 2013; van Noordwijk and de Jong 1986). According to the Individual Optimization Hypothesis (Pettifor et al. 1988), optimal clutch size is the one that maximizes the number of recruits. Apart from post-laying casualties that may reduce original clutch size, suboptimal clutch sizes arise from both intra- and interspecific brood parasitism, increasing the number of eggs beyond such a self-established optimum. On the one side, parents suffering brood parasitism might benefit from a dilution effect against predators if nest predation is partial (Hamilton 1971). However, on the other side, parents incur higher prenatal (incubation) and postnatal costs, which are well documented in interspecific parasitism,

but insufficiently in the case of intraspecific brood parasitism (reviewed in Lyon and Eadie 2008).

Costs of reproduction have gained much attention in the last decades (Reznick 1985). However, most studies have overlooked potential costs, such as parasitism, and possible buffers, such as the social mating system. In addition, data from medium- and long-term studies might unveil seemingly non-costly decisions, which are only apparent in the long-term. Therefore, comprehensive studies taking these variables into account are needed. We performed a study in the spotless starling, *Sturnus unicolor*, where we aimed to assess state- and context-dependent costs of reproduction in two consecutive breeding seasons. Starlings are an ideal species, as they are relatively long-lived social breeders, with a facultative polygynous mating system. Around 50% of individuals raise two broods per year, and reproductive effort differs significantly among individuals, independently of their mating system. Moreover, similar to European starlings, *Sturnus vulgaris*, they suffer intraspecific brood parasitism from floaters that do not have a nest (Pilz et al. 2005), which may increase incubation and rearing costs for individuals that are parasitized. We focused on reproductive costs, because long-lived species should prioritize survival over reproduction, and thus, if reproduction is costly, future reproduction is more likely to be affected than survival (Hamel et al. 2010). Indeed, most studies in birds report a cost in future reproduction, whereas a cost in survival is rarely detected, perhaps because costs in survival require much larger sample sizes for demonstrating significant effects (Lindén and Møller 1989). Therefore, in the short term, we expected to find that individuals with high investment in the first brood might refrain from breeding or defer the second brood. If high investors pay a cost, it could be apparent by longer inter-brood lags to recover from the effort. Moreover, starlings could decrease the effort in the second brood. Finally, in the long term, birds could pay the costs by skipping breeding the following year, or by delaying the onset of breeding and/or diminishing investment in the next year. The mating system might buffer costs. However, within a given mating system, we would expect that birds that have a higher investment would face a cost in the short term or the long term. Moreover, in the case a cost was detected, we wanted to know which particular component of parental investment was responsible for it; in other words, whether incubation effort (prenatal), or postnatal effort, or both were responsible for the observed cost.

Methods

Study site and study species

We used data from a long-term project on the biology of the spotless starling in a Dehesa forest north of Madrid, Spain.

The area consists of an open formation of oak, *Quercus pyrenaica*, and ash trees, *Fraxinus angustifolia*, used for cattle grazing. The climate is continental Mediterranean [Köppen–Geiger climate classification: Csb category (reviewed in Peel et al. 2007)] with hot and dry summers. 250 nest boxes are interspersed in the dehesa, and spotless starlings have been the subject of study for the last 12 years. Spotless starlings are facultatively polygynous, hole-nesting passerine birds that lay up to two broods per year. The first one between early and mid-April and the second one at the end of May (Muriel et al. 2015), investing more resources in early than in late clutches (López-Rull et al. 2010). For this study, reproductive events from four consecutive field seasons (2012–2015) were compiled. Starlings were individually marked with numbered metal bands and with a passive integrated transponder (PIT) tag (Trovan Ltd., Douglas, UK).

General data collection

Adult starlings were trapped with spring traps in their nest boxes at the beginning of the reproductive season. Birds were sexed, and identified, and if necessary, they were banded, and a PIT tag was inserted in the back under the skin between the shoulder blades. They were weighed with a precision digital pocket scale to the nearest 0.01 g (Pesola AG, Switzerland).

To correct for the fact that females' mass increases nonlinearly as the day of laying of the first egg approaches, we extracted the residuals of the log–log regression of the relationship between individual mass and days left until the laying of the first egg for that particular female. Moreover, we included tarsus length to account for structural size. We used the residuals as a body condition index. In different species, body mass or body condition is strongly positively related to egg size (Christians 2002).

Nest boxes were regularly checked to assess the onset of breeding in the dehesa. Once the first egg was laid in any given box, it was visited daily until completion of the clutch, and the egg of the day was marked with a nontoxic permanent marker. If we found more than one new egg in a nest on a given day, we assumed that one of those eggs was laid by a parasitic female, and the clutch was categorized as parasitized. Once the whole clutch was laid, the length and width of each egg were measured with a digital caliper (Mitutoyo, Japan, precision = 0.01 mm). Egg volume was calculated by the formula: $0.51 \times \text{length} \times \text{width}^2$ (Hoyt 1979). Parasitic eggs differ in volume and color from the host's eggs, and in most cases, they can be easily recognized de visu. Usually, parasitic eggs differed in volume by more than two standard deviations from the host's clutch (pers. obs.). When differences in volume and color were smaller, we considered that the parasitic egg was the one that did not match the volume

expected according to the position in the laying order. The boxes were visited again on the expected day of hatching at incubation day 11 when the nestlings were 14 days.

The identity of the breeding pair was assessed with the help of PIT tag readers that were temporarily placed at the entrance of the nest and connected to a data logger (Trovan, Spain) hidden in a plastic box outside of the nest box. Moreover, the PIT tag system allowed us to assign the nest as a monogamous or polygynous nest, according to the exclusivity of the male. Females mated with a polygynous male were assigned as primary or secondary females according to male contribution. In other words, we checked male attendance at the nest during incubation and nestling feeding. The nest where the male contributed more was considered the primary nest and that where the female contributed more, the secondary nest. The assignment was done for the first reproductive year. During the second reproductive year, because of reduction in sample size, the number of primary and secondary females was very low (17 and 10, respectively), and therefore, both were grouped in one single level (polygynous females).

Prenatal and postnatal investment

Breeding investment, the amount of resources devoted to reproduction, was divided into the early or prenatal period, and the late or postnatal period. We considered as prenatal investment the total volume of the clutch (summed volume), excluding intraspecific parasitic eggs. However, when we considered the cost of that prenatal investment, we used the summed volume of all the eggs that were incubated, including thus parasitic eggs, since these require parental investment as well. Postnatal investment was measured on day 14 when a substantial amount of care has already been provided to the nestlings. As proxies of postnatal investment we took into account number of nestlings on day 14, the hatching success (number of nestlings alive from the original clutch size, as a relative measure of the actual investment in relation with the clutch size mothers chose to lay), and the total mass of the nestlings at the nest (summed mass).

Data analysis

All the analyses were performed with the software R version 3.1.0 (R Core Team 2014). To analyze individual variability in average egg volume and clutch size within a breeding season and between different breeding seasons (different years), we calculated the intra-class coefficient of correlation with the package *rptR* (Nakagawa and Schielzeth 2010).

Second, we wanted to know what factors affected investment in the first brood. For that, we fitted mixed models with prenatal (total volume of the clutch) or postnatal (hatching success, number of nestlings at day 14, and summed mass

of nestlings at day 14) investment as the dependent variables (one at a time). We included as fixed factors the age of the mother (young = 1 year, or old = older than 1 year), to account for experience, the social mating system of the nest (monogamous, primary or secondary) as a measure of male contribution and intraspecific parasitism (presence or absence) as a factor modifying the optimal clutch and brood size of the parents. The year of study was included as a fixed factor. We included mother identity as a random factor to account for females sampled in more than 1 year.

Count data (clutch size, number of nestlings) were analyzed using a Poisson distribution and a negative binomial distribution, respectively, with a log link function. The probability of reproduction as well as hatching success were analyzed with a binomial distribution and a logit link function. For the rest of the models, we fitted a Gaussian distribution with identity link function, after ensuring that variances were homogeneous and the normality of the residuals by visually checking normal probability plots and by plotting residuals vs. fitted values, respectively. The summed mass of nestlings was squared-transformed.

Analysis of investment in the second brood and the second year followed a similar rationale. We fitted mixed models to predict what factors affected prenatal investment in the second brood (total egg volume of the clutch) and postnatal investment (number of nestlings, hatching success at day 14, and summed mass of nestlings at day 14, one at a time). As explanatory variables, we included investment in the previous brood or the previous year. For that, we calculated two investment indices for the first breeding event in a season and for the entire first year. We included in a principal component analysis (PCA), the summed volume of the clutch (including parasitic eggs), the total summed mass, and the number of nestlings. Even though the variables are somehow correlated, they capture different aspects of the investment. This was done separately for the first breeding event (in the case of second brood's models) and the whole year (in the case of second year's models). The first component in both cases was the only component with an eigenvalue > 1, and it explained 67.6 and 81.1% of the variance, respectively. We considered as explanatory variables the social mating system of the previous breeding event or the previous year, as well as whether the female had suffered intraspecific brood parasitism or not. For the models of the second year, we considered that a nest had suffered intraspecific brood parasitism if the nest had been parasitized in at least one of the previous breeding events, and we considered a female as polygynous whenever she was in such a mating system at least once in the previous breeding year. We included the age of the mother and the year as fixed factors. The identity of the mother was included as random factor.

We included in the models two-way interactions between brood parasitism and investment as well as between mating

system and investment. Whenever non-significant, they were removed from the final model (Engqvist 2005).

General linear mixed models were computed with the package *lmerTest*, and *p* values were calculated using Satterthwaite's approximation. Generalized linear mixed models were analyzed with package *lme4*.

Whenever we found a negative correlation between current and previous reproduction, we explored whether the driver of that relationship stemmed from prenatal or postnatal investment. For that, we compared a set of candidate models using the second-order Akaike's Information Criterion (AIC_c). The set of candidate models included the null model, which considers that the response is independent of the previous investment and the full model [I ~ total volume × clutch size + total mass of the nestlings + number of nestlings, previous investment (I) being the first component of the PCA]. Moreover, we included all these parameters separately in different models. We considered that models with a $\Delta AIC_c \leq 2$ were substantially supported (Burnham and Anderson 2002). We calculated the relative weight of the models and Nagelkerke's pseudo R^2 , which can be used as a measure of explained variation (Nagelkerke 1991).

Results

For this study, we used 2885 eggs stemming from 647 clutches laid by 319 females. Individual egg volume was highly repeatable within as well as between breeding seasons (within season: $R = 0.681$, CI = 0.631–0.717, $P = 0.001$; between seasons: $R = 0.767$, CI = 0.696–0.822, $P = 0.001$; Fig. 1a, b, respectively). Clutch size was repeatable within a season ($R = 0.609$, CI = 0.522–0.660, $P = 0.001$), but not significantly between years ($R = 0.005$, CI = 0–0.092, $P = 0.454$).

During the years of study, the average volume of eggs decreased ($F_{3, 433.3} = 3.321$, $n = 473$, $P = 0.019$). Mothers in a better body condition laid larger eggs ($F_{1, 452} = 5.572$, $n = 472$, $P = 0.019$). Within a clutch, egg volume significantly decreased with increasing position in the laying sequence in a non-linear way ($F_{1, 3002} = 56.841$, $n = 3770$, $P < 0.001$; Fig. 2), with eggs 1 and 2 being larger than the rest. The mating system was not significantly related to the average clutch volume ($F_{1, 417.2} = 1.440$, $n = 473$, $P = 0.237$). We did not find a significant relationship between the average volume of a clutch and clutch size ($F_{1, 628.8} = 1.703$, $n = 835$, $P = 0.192$).

Factors affecting parental investment in the first brood

We found no significant differences in female prenatal investment, measured as the total volume of the clutch, between different mating systems ($F_{2, 465.9} = 0.156$, $n = 485$,

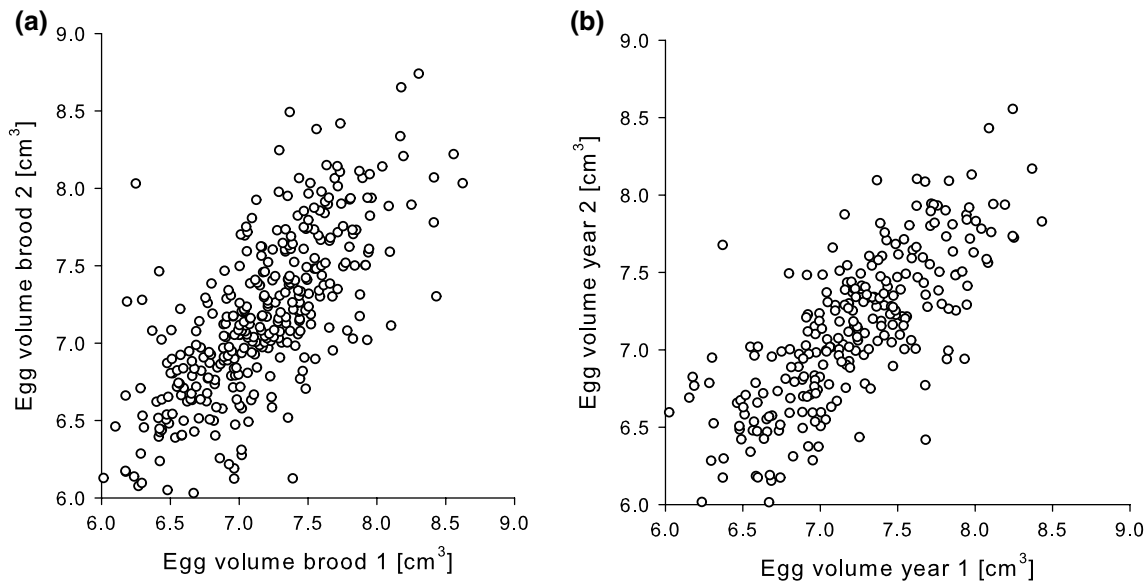


Fig. 1 Repeatability between **a** egg volume (in cm^3) in the first brood and the second brood within a breeding season and between **b** consecutive years in spotless starlings. See text for statistics

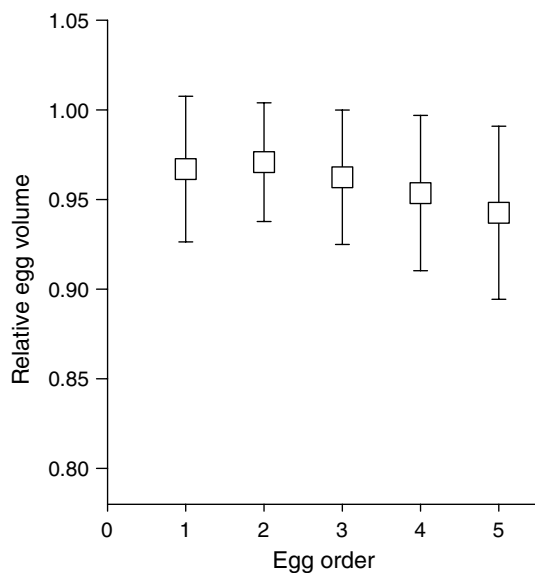


Fig. 2 Differences in egg volume in relation with laying order. As average volume among clutches differed, we calculated the relative egg volume as the difference between each egg within a clutch and the largest egg in that clutch. The plot represents the average relative egg volume in relation with laying order and the standard deviation

$P = 0.856$), and nests with more prenatal investment did not suffer more intraspecific parasitism ($F_{1,457.5} = 0.069$, $n = 485$, $P = 0.793$). Maternal age was not related to the average egg volume ($F_{1,475.3} = 1.394$, $n = 485$, $P = 0.238$).

Postnatal investment measured on day 14 was related to the mating system of the pair. Monogamous and primary females had a higher hatching success—measured

as the proportion of nestlings still alive at day 14 from the original clutch size—than secondary females (monogamous vs. primary females: $\beta = 0.398$, $\text{SE} = 0.23$, $n = 484$, $P = 0.089$; monogamous vs. secondary females: $\beta = -1.087$, $\text{SE} = 0.22$, $n = 484$, $P < 0.001$; primary vs. secondary females: $\beta = -1.485$, $\text{SE} = 0.30$, $n = 484$, $P < 0.001$). Monogamous and primary females had more nestlings on day 14 than secondary females (monogamous vs. primary females: $\beta = 0.057$, $\text{SE} = 0.08$, $n = 484$, $P = 0.436$; monogamous vs. secondary females: $\beta = -0.379$, $\text{SE} = 0.10$, $n = 484$, $P < 0.001$; primary vs. secondary females: $\beta = -0.489$, $\text{SE} = 0.13$, $n = 484$, $P < 0.001$). Moreover, monogamous and primary females raised a larger summed mass of nestlings than secondary females (monogamous vs. primary females: $\beta = 80.860$, $\text{SE} = 88.62$, $n = 487$, $P = 0.405$; monogamous vs. secondary females: $\beta = -195.973$, $\text{SE} = 89.95$, $n = 487$, $P < 0.001$; primary vs. secondary females: $\beta = -211.999$, $\text{SE} = 103.53$, $n = 487$, $P < 0.001$). In addition, older mothers had a higher hatching success, a larger number of nestlings, and a larger summed mass of nestlings than young females (hatching success: $\chi^2 = 3.908$, $n = 484$, $P = 0.048$; number of nestlings: $\chi^2 = 2.911$, $n = 484$, $P = 0.024$; summed mass: $F_{1,917.8} = 3.346$, $n = 487$, $P < 0.001$). Parents with parasitic eggs in the nest raised significantly more nestlings (hatching success: $\chi^2 = 18.236$, $n = 484$, $P < 0.001$; total summed mass: $F_{1,979.7} = 7.590$, $n = 487$, $P < 0.001$). We found a year effect ($F_{3,1455} = 2.898$, $n = 487$, $P = 0.035$), which was driven by higher postnatal investment in 2014 than in any other year.

Probability of laying a second brood

Females that invested more in the first brood (first component of the PCA) were more likely to have a second brood ($\chi^2 = 14.389, n = 478, P < 0.001$; Fig. 3). The same pattern was found in females older than 1 year with respect to first year females ($\chi^2 = 1.487, n = 478, P < 0.001$). Intraspecific parasitism was not significantly related to the probability of performing a second breeding attempt ($\chi^2 = 0.013, n = 478, P = 0.915$) or the mating system during the first brood ($\chi^2 = 4.524, n = 478, P = 0.104$).

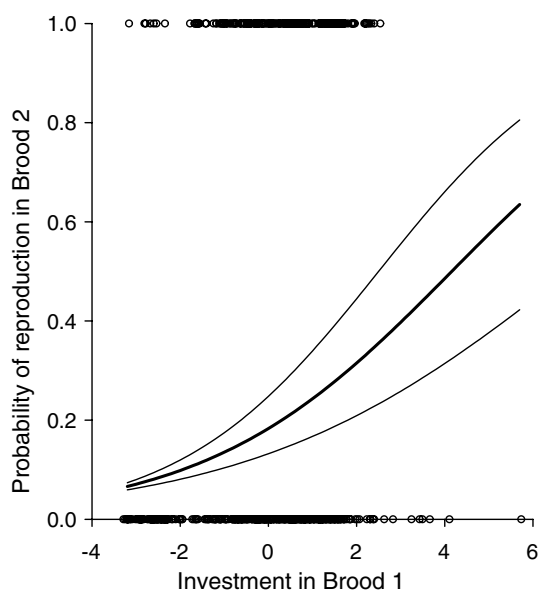


Fig. 3 Female starlings that have a higher parental investment in the first brood were more likely to lay a second clutch within a breeding season. The circles represent the actual data and the lines the predicted values and the standard deviation. See text for statistics

Factors affecting investment in the second brood

Females that invested more in the first brood not only initiated breeding significantly earlier in the second brood ($\chi^2 = 6.261, n = 224, P = 0.012$), but also had a higher prenatal investment in the second brood (total volume of the clutch: $F_{1, 203.6} = 4.236, n = 222, P = 0.040$). Moreover, females with shorter inter-clutch intervals had a larger prenatal investment in the second brood ($F_{1, 212} = 21.499, n = 222, P < 0.001$). Female age did not significantly correlate with prenatal investment in the second brood ($F_{1, 211.6} = 0.458, n = 222, P = 0.499$). Females that suffered intraspecific parasitism in the first brood did not have lower prenatal investment in the second brood ($F_{1, 187.5} = 3.827, n = 222, P = 0.052$). The mating system was not significantly correlated with prenatal investment in the second brood ($F_{2, 202.2} = 1.284, n = 222, P = 0.289$).

Investment during the first brood was not significantly related to postnatal investment in the second brood (Table 1). Females that delayed the onset of the second brood raised a significantly smaller summed mass of nestlings ($F_{1, 216.1} = 6.250, n = 226, P = 0.013$). Mating system, the age of the mother or the presence of parasitic eggs during the first brood was not significantly correlated with postnatal investment in the second brood (Table 1).

Factors affecting investment in consecutive breeding seasons

We found a year effect in the probability of reproducing in two consecutive years. Females that reproduced in 2014 were more likely to reproduce in 2015, probably because of a milder winter. We found a statistically significant interaction between the previous intraspecific brood parasitism and year. Females that suffered brood parasitism in 2014 were more likely to reproduce in 2015. For the rest of the years, the relationship was not statistically significant (Electronic

Table 1 Summary of general mixed models of postnatal investment in the second brood (hatching success, number of nestlings at day 14 and summed mass of nestlings at day 14)

	Hatching success			Number of nestlings			Summed mass of nestlings		
	Estimate (SE)	χ^2	<i>P</i>	Estimate (SE)	χ^2	<i>P</i>	Estimate (SE)	<i>F</i> (df)	<i>P</i>
Mating system		0.673	0.714		1.154	0.562		0.567 (2, 216.1)	0.568
<i>M</i> vs. <i>P</i>	0.150 (0.30)		0.626	0.151 (0.14)		0.283	101.230 (89.47)		
<i>M</i> vs. <i>S</i>	0.256 (0.42)		0.541	0.006 (0.18)		0.971	115.49 (104.77)		
<i>P</i> vs. <i>S</i>	0.405 (0.49)		0.413	0.145 (0.22)		0.508	55.066 (114.84)		
Inter-clutch interval	0.140 (0.11)	1.612	0.714	0.074 (0.04)	2.829	0.092	89.744 (52.83)	6.250 (1, 216.1)	0.013
Maternal age	0.381 (0.34)	1.226	0.268	0.010 (0.15)	0.004	0.949	84.34 (92.90)	0.142 (1, 216.1)	0.707
Brood parasitism	0.302 (0.24)	1.591	0.207	0.052 (0.12)	0.192	0.661	44.037 (82.11)	0.083 (1, 216.1)	0.773
Investment brood 1	0.049 (0.13)	0.139	0.709	0.043 (0.06)	0.451	0.502	56.232 (62.44)	0.384 (1, 216.1)	0.536

Significant effects are shown in bold

supplementary material, Table 1). None of the factors considered significantly predicted the onset of breeding the next year (Electronic supplementary material, Table 2).

In the second year, females that had suffered intraspecific brood parasitism in the previous year seemed to carry over a cost, which was apparent by the significant

Table 2 Candidate models that might explain prenatal investment (*I2*) in the second year, including the null model (*I2*·)

Candidate models	<i>K</i>	ΔAIC_c	w_i	Pseudo R^2
<i>I2</i> parasitism × postnatal _{number of nestlings}	7	0	0.422	0.120
<i>I2</i> parasitism × postnatal _{mass of nestlings}	7	0.400	0.346	0.117
<i>I2</i> parasitism × prenatal _{volume}	7	1.400	0.210	0.111
<i>I2</i> parasitism	5	7.752	0.009	0.042
<i>I2</i> prenatal _{volume}	5	8.452	0.006	0.037
Full model	10	9.274	0.004	0.099
<i>I2</i> prenatal _{volume} × clutch size	7	9.500	0.004	0.057
<i>I2</i> parasitism × prenatal _{clutch size}	7	9.600	0.003	0.057
<i>I2</i> ·	4	11.629	0.001	0.000
<i>I2</i> prenatal _{clutch size}	5	11.852	0.001	0.013
<i>I2</i> postnatal _{number of nestlings}	5	12.852	0.001	0.006
<i>I2</i> postnatal _{mass of nestlings}	5	13.052	0.001	0.005

The table presents the number of parameters present in each model (*K*), the Akaike weights (w_i), and Nagelkerke's pseudo R^2 . The models are ranked according to ΔAIC_c , and the ones in bold are the ones that received substantial support from the data ($\Delta AIC_c \leq 2$)

interaction between intraspecific brood parasitism and previous investment ($F_{1,122.5} = 9.668, n = 139, P = 0.002$). Females that made a higher than average investment in the previous year and suffered brood parasitism lowered their prenatal investment in the consecutive year (Fig. 4). The mating system they had been engaged in during the previous year ($F_{1,103.4} = 0.467, n = 139, P = 0.496$), or the age of the mother ($F_{1,129.7} = 0.020, n = 139, P = 0.886$) was not correlated with prenatal investment in the second year.

We examined those components of the previous effort that could better explain the observed cost. We decomposed investment in the first year into the prenatal (egg volume and clutch size) and postnatal parts (total number of nestlings and total mass of nestlings at postnatal day 14), and we found three models with considerable support ($\Delta AIC_c < 2$). They included the interaction of brood parasitism and an element of investment (Table 2). Considering Akaike weights, the model that included the number of nestlings to explain the observed cost was two times more likely to be the best of our candidate set of models than the one that included prenatal investment (average egg volume). In addition, the model that included the mass of the nestlings was 1.6 times more likely than the one considering prenatal investment.

Higher hatching success was attained by females that reproduced earlier in the second year (Table 3). We did not find any other significant effects of the previous investment in postnatal investment in the consecutive year (Table 3).

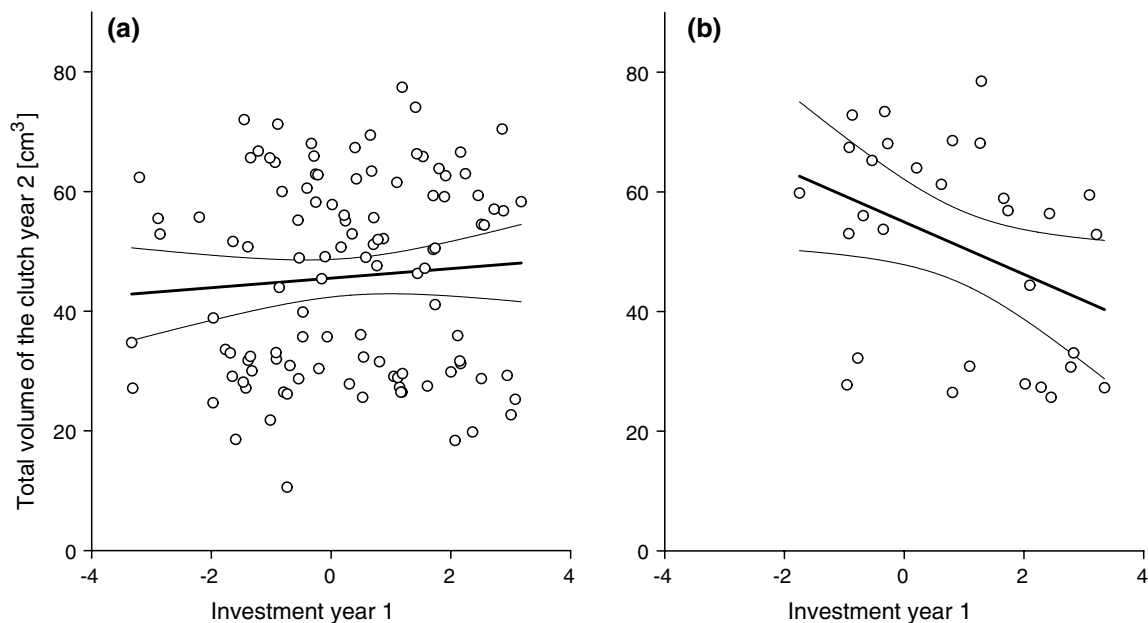


Fig. 4 Prenatal investment in the second year in relation with reproductive investment during the previous year in **a** starling females that did not suffer from brood parasitism and **b** females who suffered from

at least one parasitism event during the previous year. The circles represent the actual data and the lines the predicted values and the standard deviation. See text for statistics

Table 3 Summary of the generalized mixed models of postnatal investment in the second year (hatching success, number of nestlings at day 14) and the general mixed model of summed mass of nestlings at day 14 in the second year

	Hatching success			Number of nestlings			Summed mass of nestlings		
	Estimate (SE)	χ^2	<i>P</i>	Estimate (SE)	χ^2	<i>P</i>	Estimate (SE)	<i>F</i> (df)	<i>P</i>
Mating system		0.500	0.779		0.244	0.885	34.205 (173.79)	0.001 (1, 187.3)	0.969
Onset breeding	0.411 (0.18)	4.946	0.026	0.025 (0.04)	0.334	0.563	41.182 (43.50)	0.803 (1, 144.1)	0.372
Maternal age	0.077 (0.29)	0.072	0.788	0.004 (0.11)	0.002	0.967	56.134 (182.31)	0.009 (1, 157.9)	0.925
Brood parasitism	0.146 (0.28)	0.275	0.600	0.027 (0.10)	0.067	0.795	89.431 (174.15)	0.069 (1, 181.8)	0.792
Investment brood 1	0.033 (0.124)	0.072	0.789	0.024 (0.05)	0.275	0.600	114.538 (121.28)	0.796 (1, 185.1)	0.374

Significant effects are shown in bold

Discussion

Egg volume in spotless starlings was highly repeatable within and between breeding seasons, and it was positively related to the body condition of the female before laying. The repeatability scores (0.68–0.77) were similar to those described for other passerine species (reviewed in Christians 2002). In a given year, clutch size was also repeatable, while it changed from year to year. Moreover, we did not find a relationship between egg volume and clutch size. Indeed, in other species, it has been shown that the presence or the absence of a trade-off between egg volume and clutch size depends on maternal body condition (Kvalnes et al. 2013; van Noordwijk and de Jong 1986). For instance, tree swallow, *Tachycineta bicolor*, females in good body condition, did not trade egg volume against clutch size, whereas those in a worse body condition did (Pellerin et al. 2016).

Within a clutch, we found a non-linear decrease in egg volume with laying order: last-laid eggs were significantly smaller than earlier laid ones. In European starlings, the relation is negative too, later eggs being smaller than the average egg volume of the clutch (Slagsvold et al. 1984). Starlings start incubation before the last egg is laid, favoring hatching asynchrony (but see Nicolai et al. 2004). Moreover, smaller last-laid eggs might facilitate brood reduction, an energy-saving strategy followed by many birds that produce eggs in excess (Vedder et al. 2017).

In our study, postnatal investment in the first breeding event was not significantly related to prenatal investment, but to the mating system of the social pair and maternal age. Monogamous and primary pairs, as well as older mothers, were more successful in the first breeding event; they had higher hatching success, and they raised more nestlings with a larger summed mass. Indeed, in other species, secondary females mated with a polygynous male are usually less successful in raising a brood, whereas primary and monogamous females, with higher male attendance, have been found to be equally successful, even though males do not provision them equally (Moreno et al. 2002; Slagsvold and Lifjeld 1994; Smith et al. 1994). Therefore,

we would expect to find a difference between primary and monogamous females and secondary females in the long term. However, mating system did not correlate with investment in the second year. Other studies have shown that although females might differ in breeding performance in a given breeding season depending on their mating status, in the long term, they have similar lifetime reproductive success (Garamszegi et al. 2004), suggesting that females are able to compensate in the long run.

Moreover, in nests with intraspecific parasitic eggs, a higher proportion of eggs hatched and survived to postnatal day 14. Other studies have found opposite results. For example, in the European starling, nests with intraspecific parasitism had lower hatching success than non-parasitized nests (Evans 1988). However, our results were similar to those obtained in some brood enlargement experiments. In great tits, *Parus major*, enlarged broods fledged more nestlings than control broods, and those, in turn, fledged more offspring than nests with reduced broods. However, fledglings from control broods were as likely to become recruits as fledglings from enlarged nests (Pettifor et al. 2001), suggesting that there was a cost paid by fledglings raised in enlarged broods undetectable in the short term.

Spotless starling females with higher than average investment in the first breeding event in the season were more likely to lay a second clutch, they did not delay the onset of the second brood, and they maintained their high prenatal investment in the second clutch as well. This suggests that differences in female quality account for differences in reproductive success far and above differences in mating status (Moreno et al. 2002). Contrary to our results, barn swallows, *Hirundo rustica*, with a high investment in the first clutch had longer intervals between clutches (Møller 2007). Inter-clutch interval might serve birds to compensate for the past reproductive effort. However, animals might overcome the trade-off depending on stored reserves, resource acquisition abilities, and/or physiological state. In a recent study of black-legged kittiwakes, *Rissa tridactyla*, the reproductive effort in the season was positively related to the physiological state of the parents before laying (Merkling et al. 2017).

Furthermore, it could be argued that in a highly synchronous species such as the spotless starling, there is strong selection for breeding synchronously and this may reduce variance in inter-clutch interval.

Although we found a positive correlation between investment in the first brood and prenatal investment in the second brood, we did not find a higher postnatal investment. Second broods in our study area are usually less successful than first broods, as resource availability is lower in the second part of the breeding season (Muriel et al. 2015). A further explanation could be that some components of nestling quality might be independent of mass and number of nestlings. In barn swallows, high-quality parents needed shorter inter-clutch intervals to produce offspring with a higher immune response, which purportedly are higher quality offspring (Møller 2007). Therefore, our measure of investment based on mass and number might underestimate the actual investment.

Investment in one breeding season did not affect the probability of reproduction the following year or the onset of breeding. However, intraspecific parasitism emerged as a cost paid in the second year, independently of the mating system they were engaged in the previous year. Females with a high reproductive output that suffered brood parasitism in 1 year reduced their prenatal investment the next. Clutch or brood enlargement experiments have rendered similar results. Great tits subjected to enlarged clutch sizes in the first brood were less successful in the second brood than control parents (de Heij et al. 2006). In both cases—nests suffering from intraspecific parasitism or experimentally enlarged clutches—breeding pairs might have incurred extra incubation and rearing costs that were apparent, in our case, in the next year. Although the cost was not detected until a year later in spotless starlings, in the experiment with great tits, clutch sizes were modified by three eggs (de Heij et al. 2006), whereas a parasitic female might lay one, and less frequently two parasitic eggs in a given clutch. Black-legged kittiwakes that had a high reproductive effort during the season paid a physiological cost, for instance, in terms of lipid oxidative damage (Merkling et al. 2017). There is a time-lag between a physiological change is detected, and an observable cost in the life history of the individual, which could explain the fact that costs are usually undetected in the short term, within a breeding season, but in longer term studies.

Several studies have highlighted the costs associated with incubation (de Heij et al. 2006; Moreno and Sanz 1994), or with egg production (Nager et al. 2001), which have often been considered negligible. Although we cannot exclude incubation costs, postnatal effort in the first breeding year because of raising enlarged broods explained the lower prenatal investment in the second breeding season by parasitized pairs. This result might further support

the individual optimization hypothesis, which states that parents lay optimal clutch sizes, and deviations from that number lead to reduced fitness (Lack 1947; Leach et al. 2017; Pettifor et al. 2001).

Interestingly, investment in the second year was independent of the age of the mother. It is known that experience plays an important role in predicting reproductive success, but it does not predict the probability of reproduction in the following breeding event in all species. For instance, in black-legged kittiwakes and in wandering albatrosses, *Diomedea exulans* this relationship is lacking (Cam and Monnat 2000; Croxall et al. 1992). Whereas age and experience are usually tightly linked, they are not synonyms. In western gulls, *Larus occidentalis*, hatching success was positively related to experience, whereas fledging success was related to age (Pyle et al. 1991), which reflects how individual heterogeneity drives variation in life-history strategies and trade-offs (Moyes et al. 2008). In our study, we did not measure experience but age, and probably our proxy of individual quality did not capture the complexity of individual heterogeneity.

In conclusion, our study shows that both the state (age and previous investment) and the ecological contexts of the breeding event (i.e., intraspecific brood parasitism and mating system) of the individual are important factors in the assessment of reproductive trade-offs. Moreover, we provide support for the individual optimization hypothesis. Females are able to perform high reproductive investment without noticeable costs as long as they do not face brood enlargement because of intraspecific brood parasitism. Whether they are capable of keeping up with a high reproductive effort throughout their lifetime requires further study.

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Author contribution statement RM originally formulated the idea, RM, JM, LP-R, and DG developed methodology and conducted fieldwork, RM performed statistical analyses, and RM, JM, LP-R, APM, and DG wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

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