

## Facultative and non-facultative sex ratio adjustments in a dimorphic bird species

Simone Santoro, Andy J. Green, John R. Speakman and Jordi Figuerola

S. Santoro (<[orcid.org/0000-0003-0986-3278](http://orcid.org/0000-0003-0986-3278)>)([santoro@ebd.csic.es](mailto:santoro@ebd.csic.es)), A. J. Green and J. Figuerola, Dept of Wetland Ecology, Doñana Biological Station-CSIC, Américo Vespucio s/n, ES-41092 Seville, Spain. – J. R. Speakman, Inst. of Environmental and Biological Sciences, Univ. of Aberdeen, Aberdeen, UK.

If parental allocation to each offspring sex has the same cost/benefit ratio, Fisher's hypothesis predicts a sex ratio biased towards the cheaper sex. However, in dimorphic birds there is little evidence for this, especially at hatching. We investigated the pre-fledgling 1) sex ratio, 2) body condition and 3) sex-differential mortality in a population of the glossy ibis *Plegadis falcinellus*, in southern Spain between 2001 and 2011. We defined two age groups for the period between hatching and fledging. We also compared pre-fledgling with the autumn sex ratio. Metabolic rates were estimated by the doubly labeled water (DLW) technique to establish that sons (the bigger sex) were 18% more energy demanding than daughters, and to compute the predicted Fisher's sex ratio (0.465). As population size increased between years, body condition decreased in both sexes, and mortality increased more for daughters than sons prior to fledging. At the same time, the proportion of males among chicks close to fledging increased (average sex ratio: 0.606) while the proportion close to hatching decreased (average sex ratio: 0.434, in line with Fisher's prediction). Furthermore, the proportions of males at fledging and the following autumn were negatively correlated across years. We suggest that, as population density increased and conditions worsened the larger sex had relatively higher survival. These differences in survival produce a shift from a facultative female-biased sex ratio at hatching into a non-facultative male-biased sex ratio of fledglings. Additionally, the excess of males at fledging was counterbalanced by sex-related dispersal during the autumn. Overall, glossy ibis sex ratio is a product of a combination of facultative and non-facultative adjustments triggered by environmental conditions, driven by rapid population growth, and mediated by highly interrelated life-history traits such as body condition, mortality, and dispersal.

Differential sex allocation occurs when different amounts of resources are devoted to sons and daughters, which can bias the sex ratio, an important life-history trait, at the individual and/or population level. Even though clear-cut boundaries are not well defined (West 2009), sex ratio is usually studied at three different stages: primary (at fertilization), secondary (at birth) and tertiary (at sexual maturity). However, differential mortality or dispersal of sons and daughters can also cause shifts in sex ratio in between these stages.

Since Darwin (Darwin 1871) addressed this topic, several evolutionary theories and empirical studies have been proposed (reviewed by Hardy et al. 2002, West 2009). Fisher's landmark equal allocation theory (Fisher 1930) aimed to explain the common occurrence of balanced sex ratios in nature by postulating that, if fitness cost/benefit ratios are not sex dependent, parents should invest the same in daughters and sons. Thus, provided both daughters and sons have the same cost for parents, frequency-dependent selection would offset every deviation from parity and lead to a balanced, evolutionary stable sex ratio. On the other hand, when the sexes require different amounts of resources, selection would skew the sex ratio towards the cheaper sex, if identical parental allocation for each sex was maintained.

Other theories have been developed by considering situations where there are different costs and benefits of male and female offspring, when sex allocation should be adjusted to maximize fitness. There are two main theoretical frameworks: 1) one postulating that parents should adjust offspring sex ratio depending on predictable sibling cooperation or competition (local mate competition; Hamilton 1967, local resource competition; Clark 1978, Taylor and Bulmer 1980, or local resource enhancement; Emlen et al. 1986, Lessells and Avery 1987), and 2) one proposing that adaptive sex ratio adjustments depend on the specific conditions of parents (Trivers and Willard 1973). In birds, although a number of alternative explanations coexist (reviewed by West 2009), linear (Wiebe and Bortolotti 1992, Appleby et al. 1997, Arroyo 2002) or non-linear (Millon and Bretagnolle 2005) relationships between environmental covariates and sex ratio have often been interpreted as supporting the Trivers and Willard hypothesis.

Given the central role offspring costs play in sex allocation theories, dimorphism has often been considered a proxy for different rearing costs (Trivers and Hare 1976, Clutton-Brock et al. 1985, Magrath et al. 2007), and sexually size dimorphic species have been used as models

for testing sex allocation theories (Stamps 1990, Anderson et al. 1993, Torres and Drummond 1999). Nevertheless, the relationship between sexual size dimorphism and differential costs has rarely been demonstrated (but see Magrath et al. 2007). In species with parental care, sex allocation refers to the resources (time and energy) parents devote to their offspring (Komdeur 2012). As well as dimorphism, differential mortality during the parental care period can alter the costs of each offspring sex, provoking facultative modification in primary sex ratio but also facultative and non-facultative sex ratio variation at the time of fledging (Leigh 1970, West 2009). For instance, if offspring of the larger sex suffer higher mortality then the difference in raising costs between the larger and smaller sex would be reduced (because of shorter parental care time, Komdeur 2012). This might explain why, in dimorphic birds, there is more support for Fisher's prediction at fledging than at hatching (Pen et al. 2000, Benito and González-Solis 2007). Also, if differential mortality is determined by environmental factors and not by facultative manipulation, the sex ratio at fledging can be biased in the opposing manner to that predicted by theory (Dhondt and Hochachka 2001). In such a scenario, sex-related dispersal could provide a facultative mechanism to offset a maladaptive sex ratio.

The body condition of chicks can be related to food availability and/or quality, competition for resources and parasite load. While a direct relationship with these factors can be difficult to be ascertain, these processes can be mediated by population density (reviewed by Brown and Brown 1996). Two opposing hypotheses have been postulated to explain how population density may affect access to resources (reviewed by Brown and Brown 2001): 1) the enhanced foraging efficiency hypothesis, which predicts that at high density the access to resources is facilitated by some behavioral change (Ward and Zahavi 1973, Allainé 1990), and 2) the increased competition hypothesis which posits that, as population size increases, foraging efficiency declines (Tella et al. 2001, Dann and Norman 2006). Body condition of chicks can also depend on increasing parasite load (Merino and Potti 1995) which can increase along with colony size (Brown and Brown 1986, Rózsa et al. 1996). According to the 'silver spoon' hypothesis (Grafen 1988), conditions at an early life-stage affect the individual fitness, as confirmed by many empirical studies (Stamps 2006, Van de Pol et al. 2006). Thus, following the above rationale, the body condition of chicks might change the sex-related costs and benefits (Kruuk et al. 1999, Wilkin and Sheldon 2009), and therefore cause adaptive sex ratio adjustments.

The present study was centered on sex ratio variation and its underlying causal mechanisms in the glossy ibis *Plegadis falcinellus*, a dimorphic species nesting in Doñana (south-western Spain) (Santoro et al. 2010) where it has undergone an explosive population expansion from seven pairs in 1996 to more than 7000 pairs by 2011. Only population-level data on sex-ratios were available because the chicks, cared for by both parents, leave the nest and form creches when they are two weeks old (Cramp and Simmons 1977) and collection of brood-level data at an earlier stage was not feasible because of potential disturbance to the breeding colony (Götmark 1992).

We aimed to investigate: 1) if male chicks had greater energetic requirements than females, as expected from their larger size at fledging, 2) if pre-fledgling sex ratio departed from that expected from simple chromosomal assortment (1:1) or from Fisher's equal allocation theory (according to the estimated energetic requirement of each sex), 3) if pre-fledgling sex ratio, sex-specific body condition or sex-differential mortality depended on colony size or other proxies for the availability of resources, and 4) if the pre-fledgling and autumn sex ratios were inversely related as expected if dispersal counterbalances the excess of one sex amongst offspring. Since time elapsed from hatching can affect the proportion of males by differential mortality, we tested the sex ratio hypotheses by considering pre-fledglings either as a whole or divided into two subgroups according to their age.

## Methods

### Study area, field data

The 'Lucio de la FAO' ("FAO" hereafter) hosts the main glossy ibis colony at Doñana, a protected area in south-western Spain (Santoro et al. 2010, Ramo et al. 2013). Here, seven pairs settled in 1996 and since then this species has regularly nested in the area, except for three years (1999, 2005 and 2012) when drought events prevented them from breeding owing to an absence of foraging habitat. At the FAO colony, the number of breeding pairs has increased dramatically (more than 2000 pairs in 2011) and since 2004, additional breeding sites were occupied by this species in Doñana, hosting about 4000 pairs in 2011 (Ramo et al. 2013).

Chicks were molecularly sexed (Griffiths et al. 1998) from samples obtained during ringing operations at the main colony (Santoro et al. 2010). Samples were obtained by either 1) pulling of one outer rectrix or 2) taking a sample of blood from the medial metatarsal vein.

### Statistical analyses

If sex-differential mortality occurred during the parental care period, then we would expect to find a different sex ratio at hatching and at fledging, and therefore observe differences in sex-ratio between younger and older pre-fledglings. For this reason, we defined two subgroups according to age. As a proxy for an individual's age regardless of sex, we used the wing length, a non-dimorphic measure in this species (Figueroa et al. 2006). Wing length has been shown for other bird species to be relatively unaffected by nutritional status and a good proxy for age of chicks (Gaston 1985, Benowitz-fredericks and Kitaysky 2005). We considered the younger group to be those whose wing length was equal to or shorter than the overall median (sexes combined). Then, ecological hypotheses on sex ratio variation were tested using pre-fledgling sex ratio for the two age groups separately as well as on the whole data set.

All statistical analyses in this study were implemented in R ver. 3.0.2. The sample size and the number of degrees of freedoms for each hypothesis tested differed according to the data available for the specific set of variables.

## DNA-sampling procedure: effect on sex ratio and body condition

Whereas pulling a feather is a straightforward task and requires a short handling time, bleeding a chick is a more time demanding (Magrath et al. 2007) and specialized task providing a larger blood sample suitable for other studies. Thus, a systematic tendency to avoid bleeding smaller or less healthy-looking individuals may exist. To check if this was a source of bias for our study, we compared the sex ratio and body condition observed in chicks that were bled against those from which a feather was pulled. We assumed the latter to be completely random with respect to sex and body condition, because all chicks encountered outside nests were ringed, and feathers were taken from as many of these as time allowed.

As a body condition index, we used the scaled mass index (SMI) which has several advantages over alternative methods (Peig and Green 2009, 2010), and which performs well in birds (Bókony et al. 2012, Guillemain et al. 2013). Given that glossy ibis chicks are sexually dimorphic (Figueroa et al. 2006), the SMI was calculated separately for each sex using measures of body mass and tarsus length (details in Supplementary material Appendix 1).

First, to test the hypothesis that sampling by bleeding biased the sex ratio estimates, we considered a subset of our data drawn from colony visits ('field-sessions' hereafter) in which both sampling procedures were used ( $n_{\text{feather}} = 1080$ ,  $n_{\text{bleeding}} = 597$ ,  $n_{\text{sessions}} = 24$ ,  $n_{\text{years}} = 7$ ). We ran a binomial GLMM (*glmer* function from package *lme4*; Bates et al. 2012) considering the sex ratio (number of males out of total number of sampled chicks) as response variable (*cbind* function; Wilson and Hardy 2002), the year as a random factor and the sampling procedure as an explanatory variable. We also aimed to rule out the possibility that the effect of sampling procedure on sex ratio was due to a procedure-related propensity to select individuals of different ages, for which different sex ratios might exist. Thus, we ran a binomial GLMM considering the sex of an individual sex as response variable, field-sessions nested within year as random effects and the sampling procedure and wing length (a proxy for age) as predictors together with their interaction. We used a subset of data from years for which both sampling procedures had been used, and wing length was measured ( $n_{\text{feather}} = 637$ ,  $n_{\text{bleeding}} = 459$ ,  $n_{\text{sessions}} = 8$ ,  $n_{\text{years}} = 5$ ).

Secondly, to test the effect of sampling procedure on the body condition index we performed a gaussian LMM (*lme* function from package *nlme*; Pinheiro et al. 2013) on a subset of data for which both bleeding and feather samples were available in each field-session ( $n_{\text{feather:females}} = 321$ ,  $n_{\text{feather:males}} = 316$ ,  $n_{\text{bleeding:females}} = 174$ ,  $n_{\text{bleeding:males}} = 285$ ,  $n_{\text{sessions}} = 8$ ,  $n_{\text{years}} = 5$ ). We considered the average body condition of each sex for each field-session and sampling procedure as the response variable, year as a random factor, and sex, sampling procedure and their interaction as predictors. The LMM was weighted for the number of replicates from which average values of body condition were calculated for each field-session.

## Energetic requirements of male and female chicks

Daily energy expenditure (DEE) was estimated using the DLW technique (Butler et al. 2004). We fenced a small section of the colony (50 × 50 m) using a plastic wire mesh (1.5 m high) from 20 May to 25 May 2009. Forty-six chicks (20 females and 26 males) were injected intraperitoneally with 0.5 ml of  $^2\text{H}$ - and  $^{18}\text{O}$ -enriched water (Speakman 1997). All the dose syringes were weighed to four decimal places prior and post administration using an electronic precision balance. After being injected, chicks were retained in shaded and ventilated cardboard boxes during an hour, to allow isotopes to reach equilibrium (Król and Speakman 1999), then a first blood sample was taken and the chicks were released inside the fenced area. A second blood sample was taken after 48/72 h (fieldwork sessions on 20, 22 and 25 May) to estimate the isotope elimination rate and minimize the influence of circadian deviation in DEE (Speakman and Racey 1988). Taking samples over multiple days minimizes the substantial day to day variance in DEE (Speakman et al. 1994, Berteaux et al. 1996). Four individuals were blood-sampled without prior injection to estimate background isotope enrichments of  $^2\text{H}$  and  $^{18}\text{O}$  (Speakman and Racey 1987; method D). Blood samples were immediately heat sealed into 2 × 100 µl glass capillaries. Capillaries that contained the blood samples were then vacuum distilled (Nagy 1983), and water from the resulting distillate was used to produce  $\text{CO}_2$  and  $\text{H}_2$  (methods in Speakman et al. 1990 for  $\text{CO}_2$  and Speakman and Król 2005 for  $\text{H}_2$ ). The isotope ratios  $^{18}\text{O}$ :  $^{16}\text{O}$  and  $^2\text{H}$ :  $^1\text{H}$  were analyzed using gas source isotope ratio mass spectrometry. Samples were run alongside three lab standards for each isotope (calibrated to International standards) to correct delta values to ppm. Isotope enrichments were converted to values of daily energy expenditure using a single pool model as recommended for this size of animal by Speakman (1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser and Schekkerman 1999). We chose the assumption of a fixed evaporation of 25% of the water flux (equation 7.17: Speakman 1997), which has been established to minimize error in a range of conditions (Visser and Schekkerman 1999, Van Trigt et al. 2002).

No knowledge about potential differences in fledging times between sexes exists in this species, and samples were collected from chicks of different ages. For these reasons, we assumed differences between sexes in estimated DEE reflected differences in total expenditure, and considered them as proxies of investment in sons and daughters (Magrath et al. 2007).

We tested for sexual differences in DEE using linear regression models with type III sum of squares (function *lm* from package *car*). We used DEE as response variable and sex and wing, or sex and body mass, as predictors. Unlike wing length, body mass is highly dimorphic among glossy ibis chicks (Figueroa et al. 2006).

## Pre-fledgling sex ratio: departure from parity and Fisher's hypothesis

According to Fisher's argument, natural selection favors equal allocation in both sexes, hence, it is expected that:

$$nF \times \bar{cF} = nM \times \bar{cM} \quad (1)$$

Where  $\bar{cF}$  and  $\bar{cM}$  represent the average costs of raising one female and one male respectively, and  $nF$  and  $nM$  the number of males and females (adapted from Charnov 1982). By simple algebra, it can be shown that predicted sex ratio according to Eq. 1 is:

$$\frac{nM}{nM + nF} = \frac{\bar{cF}}{\bar{cF} + \bar{cM}} \quad (2)$$

Thus, in the case where the average costs of rearing a female and a male are identical  $\bar{cF} = \bar{cM}$ , the expected sex ratio equals 0.5 and Fisher's argument cannot be distinguished from simple meiotic sex determination (Griffiths et al. 1998, Harvey et al. 2006). On the contrary, if  $\bar{cF} \neq \bar{cM}$ , then the predicted sex ratio is not balanced and its value can be estimated by Eq. 2.

We used the average DEE value of females and males as proxies for the average costs of raising daughters and sons respectively, to calculate the predicted sex ratio according to Fisher's equal allocation theory (Eq. 2). Then, we computed yearly sex ratio values as the proportion of male fledglings within the total sample of sexed chicks.

We tested if the sex ratios observed in different years deviated from those predicted according to the Fisher' and random assortment hypotheses by using a one-sample t-test on the yearly differences between observed and predicted sex ratios.

### Environmental covariates and relationship with pre-fledgling sex ratio, body condition and sex-differential mortality

We examined the correlations between breeding population size or the surface area of flooded marshes on the one hand, and the pre-fledgling sex ratio, body condition of each sex, and sex-differential mortality on the other. Marsh area is a measure of the amount of feeding habitat available (details in Santoro et al. 2010), and is dependent on winter rainfall which itself has a positive effect on population growth (Ramo et al. 2013). A preliminary analysis showed that, whereas the yearly average wing length of male chicks increased linearly from 2002 to 2011 ( $t = 4.2$ ,  $DF = 5$ ,  $r = 0.88$ ,  $p < 0.05$ ), that of females did not ( $t = 0.98$ ,  $DF = 5$ ,  $r = 0.40$ ,  $p = 0.38$ ). Thus, assuming that there was no bias from the research staff towards collecting data from older individuals as time progressed, we used the yearly average difference between wing lengths of male and of female chicks as a proxy of sex-differential mortality ('sex-biased mortality' hereafter). Additionally, in cases where we found a significant correlation between body condition and other variables, we tested for a difference between sexes by comparing the two slope coefficients with the Z-test (Cohen et al. 2003). We did the same to compare slopes between age groups when there was a significant correlation between sex ratio and colony size.

### Pre- and post-fledgling sex ratios

We tested the correlation between pre- and post-fledgling sex ratios. Since we aimed to test if the sex ratio close to the fledging time was inversely related to the autumn sex ratio,

we tested this relationship on all the pre-fledglings (regardless of age) and those closer to fledging. As estimates of post-fledgling sex ratio, we used estimates from capture-recapture analyses performed in Santoro et al. (2013) on individuals first resighted between October and December of each year.

## Results

### Effect of DNA sampling procedure on sex ratio and body condition

The probability that an individual was a male was higher among those whose DNA was obtained by bleeding than those which were feather-sampled (slope  $\pm$  SE =  $0.5 \pm 0.1$ ,  $z = 4.78$ ,  $p < 0.001$ ). After controlling for the age effect proxied by wing length, the sampling procedure still had an effect on sex of individuals sampled (wing, slope  $\pm$  SE =  $0.40 \pm 0.065$ ,  $z = 6.23$ ,  $P < 0.001$ ; sampling procedure, slope  $\pm$  SE =  $0.34 \pm 0.12$ ,  $z = 2.91$ ,  $p < 0.01$ ; sampling procedure:wing, slope  $\pm$  SE =  $0.19 \pm 0.13$ ,  $z = 1.44$ ,  $p = 0.15$ ). However, we did not find any effect of sampling procedure on body condition estimates after controlling for the effect of sex (sampling procedure, slope  $\pm$  SE =  $0.02 \pm 0.03$ ,  $t = 0.64$ ,  $p = 0.56$ ; sex, slope  $\pm$  SE =  $-0.06 \pm 0.03$ ,  $t = -1.94$ ,  $p < 0.05$ ; sampling procedure: sex, slope  $\pm$  SE =  $-0.003 \pm 0.05$ ,  $t = -0.07$ ,  $p = 0.94$ ). To avoid any bias due to sampling procedure, further analyses on sex ratio and on sex-differential mortality were performed using only individuals sexed from feather samples.

### Daily energy expenditure of female and male chicks

Estimated DEE was higher in males than in females (mean  $\pm$  SD:  $510.36 \pm 15.2$  kJ day<sup>-1</sup> versus  $442.96 \pm 13.3$  kJ day<sup>-1</sup>) after controlling for wing size (sex, estimate  $\pm$  SE =  $67.40 \pm 20.52$ ,  $F_{1,43} = 10.78$ ,  $p < 0.01$ ; wing, slope  $\pm$  SE =  $1.39 \pm 0.64$ ,  $F_{1,43} = 4.7$ ,  $p < 0.05$ ; Fig. 1). However, such differences disappeared when controlling for body mass (sex, slope  $\pm$  SE =  $21.64 \pm 20.95$ ,  $F_{1,43} = 1.07$ ,  $p = 0.31$ ; body mass, slope  $\pm$  SE =  $0.74 \pm 0.16$ ,  $F_{1,43} = 21.8$ ,  $p < 0.001$ ), which was the best predictor of DEE regardless of individual sex.

### Observed sex ratio against predictions from Fisher's and random assortment

According to Eq. 2 and average DEE values for female and male chicks, the expected sex ratio according to Fisher's equal allocation theory is 0.465, i.e. skewed towards females. However, over the study period the overall pre-fledging sex ratio was on average male-biased (0.523). This observed sex ratio was significantly higher than that predicted by Fisher ( $t = 4.42$ ,  $DF = 8$ ,  $p = 0.002$ ), but not from that predicted by random assortment ( $t = 1.72$ ,  $DF = 8$ ,  $p = 0.124$ ). However, for the younger chicks, sex ratio was on average female-biased (0.434), and significantly lower than the balanced sex ratio ( $t = -3.04$ ,  $DF = 6$ ,  $p = 0.023$ ), but not different from that predicted by Fisher's hypothesis ( $t = -1.44$ ,  $DF = 6$ ,  $p = 0.201$ ). On the contrary, for the older chicks the observed

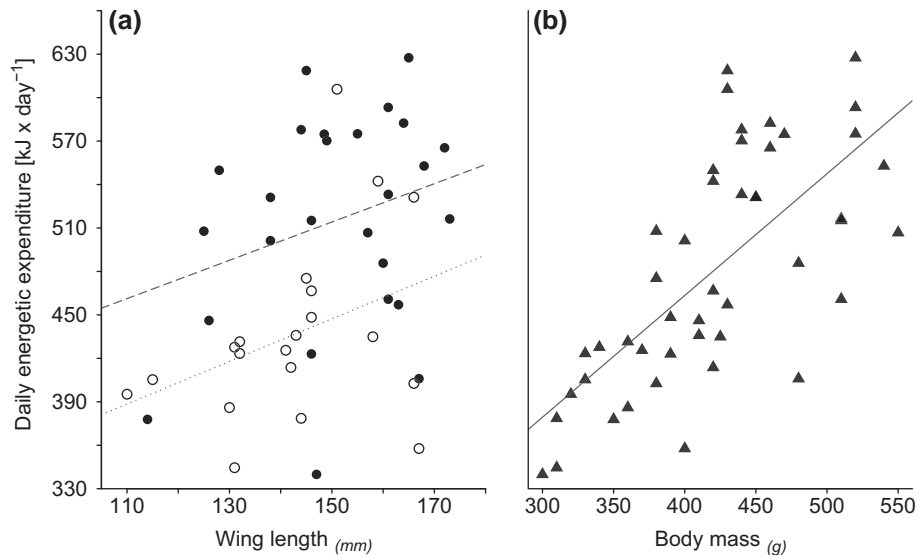


Figure 1. Metabolic costs of female and male chicks. (a) Linear relationship of female (empty circles – dotted line) and male (full circles - dashed line) chick wing length with daily energy expenditure (DEE) as estimated by a doubly labelled water experiment. (b) Linear relationship of chick (regardless of sex) body mass and DEE.

sex ratio was on average male-biased (0.606) and deviated significantly from both a balanced ( $t = 3.72$ ,  $DF = 6$ ,  $p = 0.010$ ) and Fisher's sex ratio ( $t = 4.95$ ,  $DF = 6$ ,  $p = 0.003$ ).

### Relationship between environmental covariates and pre-fledgling sex ratio, body condition and sex-differential mortality

The size of the breeding population of glossy ibis between years at the FAO colony was positively correlated with the overall proportion of males ( $t = 3.47$ ,  $DF = 7$ ,  $r = 0.80$ ,  $p < 0.05$ ) and with sex-biased mortality ( $t = 3.63$ ,  $DF = 5$ ,  $r = 0.85$ ,  $p < 0.05$ ) among pre-fledglings. However, by considering the age groups, we found that with increasing colony size the proportion of males among younger chicks decreased ( $t = -2.55$ ,  $DF = 5$ ,  $r = -0.75$ ,  $p = 0.05$ ), while the opposite occurred among older chicks ( $t = 1.98$ ,  $DF = 5$ ,  $r = 0.66$ ,  $p = 0.10$ ) (Fig. 2). This difference in sex ratio vs. colony size slopes between age groups was highly significant ( $z = 3.22$ ,  $p = 0.001$ ).

In contrast, the size of the breeding population was negatively correlated with body condition (females:  $t = -2.96$ ,  $DF = 8$ ,  $r = -0.72$ ,  $p < 0.05$ ; males:  $t = -4.11$ ,  $DF = 8$ ,  $r = -0.82$ ,  $p < 0.01$ ), with no difference in this effect between sexes ( $z = 1.31$ ,  $p = 0.19$ ) (Fig. 3).

The surface area of flooded marshes was not related to overall sex ratio ( $t = 1.21$ ,  $DF = 7$ ,  $r = 0.42$ ,  $p = 0.26$ ), sex ratio of younger pre-fledglings ( $t = 0.14$ ,  $DF = 5$ ,  $r = 0.06$ ,  $p = 0.89$ ), sex ratio of older pre-fledglings ( $t = 1.98$ ,  $DF = 5$ ,  $r = 0.25$ ,  $p = 0.58$ ), sex-biased mortality ( $t = 1.22$ ,  $DF = 5$ ,  $r = 0.48$ ,  $p = 0.27$ ), female body condition ( $t = -1.88$ ,  $DF = 8$ ,  $r = -0.55$ ,  $p = 0.10$ ) or male body condition ( $t = -1.42$ ,  $DF = 8$ ,  $r = -0.45$ ,  $p = 0.19$ ).

### Relationship between pre- and post-fledgling sex ratio

We found that an increasing proportion of males among pre-fledglings was associated with a decreasing proportion

of males among post-fledglings about four months later ( $t = -2.39$ ,  $DF = 7$ ,  $r = -0.67$ ,  $p < 0.05$ ). Although the sample size available was reduced by limiting the test to older pre-fledglings, the effect size of this relationship increased ( $t = -3.15$ ,  $DF = 5$ ,  $r = -0.82$ ,  $p < 0.05$ ) (Fig. 4).

## Discussion

By considering younger and older pre-fledglings, we found sex ratio patterns that would have gone undetected had we not accounted for age differences. In particular: 1) younger pre-fledglings showed a significant female-biased sex ratio,

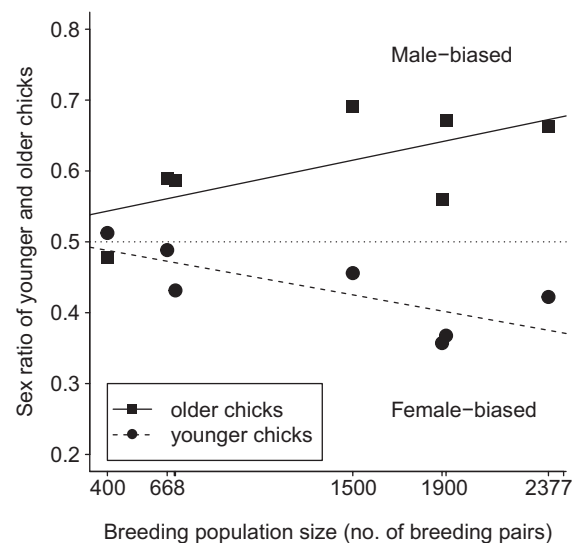


Figure 2. Sex ratios of younger and older chicks plotted against breeding population size. Black circles and the dashed regression line refer to the sex ratio of younger chicks. Black squares and the solid regression line refer to older chicks. The dotted line indicates an even sex ratio.

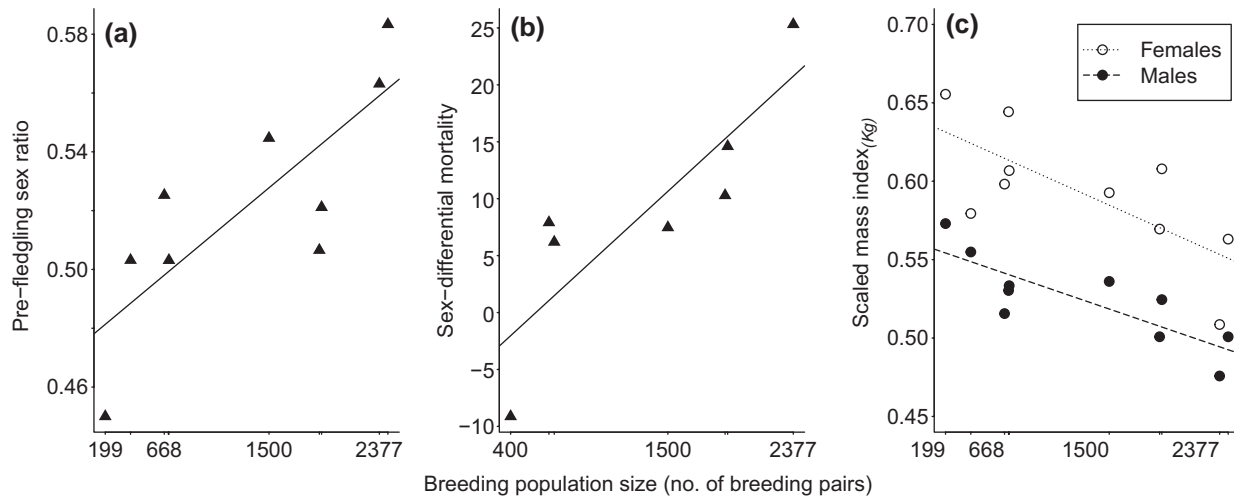


Figure 3. Linear relationships between breeding population size and: (a) pre-fledgling sex ratio, (b) sex differential mortality and (c) body condition. Pre-fledgling sex ratio is estimated as the yearly average sex ratio (proportion of males). Sex differential mortality is proxied by the yearly difference between the average wing length of male and of female chicks. Body condition estimates were calculated as the scaled mass index of mass versus tarsus, separately for females (empty circles) and males (full circles). Regression lines show the relationship between body condition and breeding population size for each sex.

which agreed with the Fisher's prediction and was enhanced by colony size increase, whereas 2) older pre-fledglings showed a significant male-biased sex ratio and the effect of colony size was the opposite to that recorded for younger birds. Overall, our findings suggest that poor conditions triggered non-facultative sex-differential mortality, leading to maladaptive fledgling sex ratios which in turn were balanced by sex-related post-fledgling dispersal. Our study suggests the co-existence of facultative and non-facultative adjustments triggered by environmental variation, and affecting life-history traits like body condition, differential mortality and sex ratio.

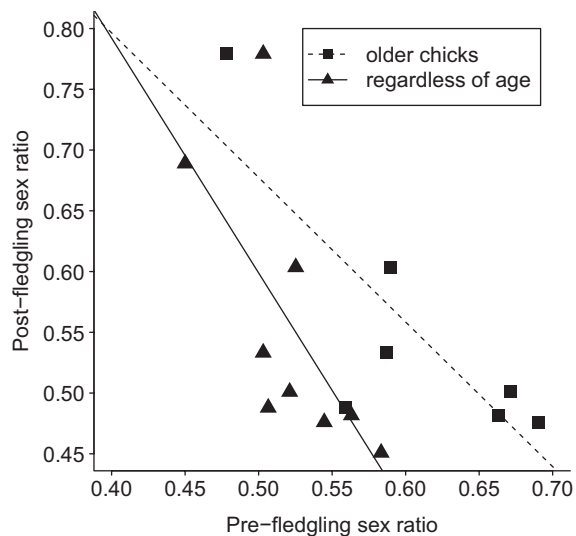


Figure 4. Relationship between pre- and post-fledgling sex ratio (proportion of males). Post-fledgling estimates are from a previous capture-recapture analysis published in Santoro et al. (2013) and refer to a few months later (May–July versus October–December). Both the linear relationship with the sex ratio of pre-fledglings regardless of age (triangles, solid line), and with the sex ratio of older pre-fledglings (squares, dashed line) are shown.

### Pre-fledgling sex ratio

If parents manipulate sex ratio during the parental care period, then Fisher's expectations apply at the time of fledging (discussed in Komdeur 2012). This is coherent with two comparative analyses on birds with sexual size dimorphism, which showed that population-level sex ratios tend to be biased towards the smaller sex at fledging (Pen et al. 2000, Benito and González-Solis 2007). However, in our study the sex ratio close to fledgling showed the opposite pattern.

The observed male-bias in sex ratio at fledging might be adaptive if, as the population increased, the cost/benefit ratio of sons over daughters also increased. Thus, parents might skew the sex ratio by changing the sex-related order (Badyaev et al. 2002) or by allocating more to sons than to daughters so enhancing higher mortality rates of the latter. However, none of these mechanisms is supported by our findings. On the contrary, we attribute the sex ratio observed close to fledging to non-facultative increased mortality of daughters over sons, determined by environmental variation. There are three lines of evidence that support for this hypothesis. First, it could be argued that the change in wing length difference between sexes, which we assumed to be a product of sex-differential mortality, may be explained by an active change by parents of the proportion of males amongst first-hatched offspring. However, this alternative explanation is completely inconsistent with the increasing proportion of females close to hatching observed in relation to density, and the simultaneous opposing pattern close to fledging. Second, if differences in mortality were due to parental decisions allocating more to sons than to daughters, we would have expected a sharper decline in body condition as colony size increased in females than in males. However our data show an identical slope for each sex. Third, if facultative manipulation of sex ratio occurs, it should be towards the same sex both at hatching and fledging (West 2009, Komdeur 2012), and this was not the case in our study.

In a similar result to ours, great tit *Parus major* showed a bias towards the larger sex at fledging under poor conditions, which was the opposite of that expected under Fisher's hypothesis (Dhondt and Hochachka 2001). The authors argue that, whereas parents might be able to control primary sex ratio, they could not control the fledgling sex ratio. Thus, a parent-offspring conflict may arise under specific conditions, with the larger offspring sex outcompeting the smaller sex, even though this is detrimental for parents' fitness. Furthermore, the average cost of females would be further reduced because of reduced parental care time devoted to this sex (discussed in West 2009), while the average benefit of males might decrease because poor condition may have a more negative impact on the fitness of the larger sex (Millon et al. 2011). If sex-related mortality occurs regardless of parental manipulation, we should then expect an increasing bias towards females at hatching as environmental conditions deteriorate. The same outcome would be expected under Fisherian frequency-dependent selection according to which the rare sex has an intrinsically higher reproductive value (Hardy et al. 2002, West 2009). Our study provides strong support for this prediction.

Furthermore, the inverse relationship we found between the fledgling and autumn sex ratios suggests that the excess of males at the end of parental care was counterbalanced by their higher dispersal rate. Our autumn sex ratio estimates originate from a previous capture-recapture analysis (Santoro et al. 2013) and refer to the proportion of males among first-resighted individuals, most of which were juveniles. The same pattern would have been observed if members of the majority sex had experienced a higher post-fledgling mortality. However, while it is uneasy to find a sound explanation for the sex- and density-related post-fledgling mortality, it is reasonable to hypothesize that the majority sex dispersed at a higher rate in order to limit intra-sexual competition for mates. This hypothesis is also consistent with the higher recruitment rate of first-year individuals found in another study on this population (Santoro 2014). It is known that the body condition of juveniles is related to their dispersal propensity (Cristol et al. 1999, Barbraud et al. 2003). Thus, a possible explanation for our result is that when body condition of both sexes was high (at low population density), females had a higher propensity to disperse than males, whereas when condition was low, the larger size of males conferred a dispersal advantage over females (Cristol et al. 1999).

Although we have referred to the effect of breeding population size, this was highly correlated to the elapsed number of years since the colony was first established, which may potentially affect other ecological processes (Duckworth and Badyaev 2007) whose effects cannot be separated from those of colony size. Our interpretations rely on an extensive literature linking density-dependence mechanisms to sex allocation patterns (reviewed by Wilson and Hardy 2002, West 2009).

In this study we suggest that the higher dispersal rate of males was a consequence of non-facultative male-bias in sex ratio at fledging. This is not consistent with the local resource competition hypothesis (Clark 1978, Taylor and Bulmer 1980) according to which the higher dispersal propensity of males would have been a cause of facultative sex ratio biasing. However, it is noteworthy that under both premises the

fitness of male offspring and parents would be enhanced as a result of reduced competition among relatives. Furthermore, the suggested relationships between deteriorating environmental conditions and sex-related differential mortality and dispersal pose intriguing questions concerning the role of sex ratio balancing within metapopulation dynamics.

## Body condition

Early life body condition is known to affect life-history traits of diverse taxa (Stamps 2006) including waterbirds (Cézilly et al. 1995, Barbraud et al. 2003). Due to the sexual size dimorphism of this species (Figuerola et al. 2006), body condition was estimated separately for each sex. Therefore relative differences in SMI between males and females should not be interpreted as sexual differences in body condition (Fig. 1; see Peig and Green 2009, 2010 for more discussion). The similar decline in body condition over time suggests that poor environmental conditions affected both male and female offspring in a similar manner. Density-dependence effects observed on glossy ibis body condition may have been caused by higher rates of disease or parasite loads (reviewed by Poulin 2011), by increasing sibling competition or by food depletion (Brown and Brown 1996, Tella et al. 2001, Forero et al. 2002, Dann and Norman 2006). The ultimate cause for the decline in body condition, as well as the mechanism responsible for the greater mortality rate in female chicks, are interesting subjects for future research. However, it would be necessary to focus on the different strategies parents may use when faced with poor conditions and, consequently, study sex ratio at the brood-level. Although the larger sex has greater nutritional requirements, and consequently tends to suffer higher mortality (Clutton-Brock et al. 1985, Bortolotti 1986, Arroyo 2002), the opposite pattern can be true if the larger sex outcompetes the smaller when resources are limited (see also Oddie 2000), as has been suggested for the closely related white ibis *Eudocimus albus* (Adams and Frederick 2009). In addition, as found in an experimental study on kestrels *Falco tinnunculus* (Fargallo et al. 2002), the smaller sex may have lower cell-mediated immunity and therefore higher disease susceptibility under food restriction.

## Energetic requirements of female and male chicks

Male glossy ibis chicks had higher energetic requirements than females. When mass was used as a covariate in the analysis, this sex effect disappeared, showing that the sex difference in size caused the difference in energy demands. This dominance of size effects on energy demands of different sexes is consistent with studies of other dimorphic species (Scantlebury et al. 2006). Since the DLW experiment was performed on individuals of various ages, we consider our assumption that the ratio between DEE of males and females is representative of the energetic demand throughout the parental care period to be realistic.

## Sampling procedure, a warning for colonial species

Working with colonial species is particularly challenging because of the potential disturbance the fieldwork can cause (Perennou et al. 1996). When collecting our data we were

initially unaware of the biases involved in bleeding. With hindsight, it is perhaps not surprising that a research team may unwittingly select larger individuals for a more intense handling procedure. A preference for larger individuals is also likely caused by the greater ease of finding the medial metatarsal vein in these individuals. As a result, DNA samples from bleeding provided a distorted sex ratio biased towards males which most likely was caused by a selection towards larger, not necessarily older, individuals. This issue may have been overlooked in previous avian studies in which chicks are sexually dimorphic and form crèches (especially other colonial birds), and caution is required when interpreting any results on sex ratio that come from blood samples in such birds.

## Final remarks

Thanks to an intensive monitoring of a newly established breeding colony, we found substantial support for facultative and non-facultative sex ratio adjustment driven by a rapidly changing population size in glossy ibis. In particular, colony size variation triggered a cascade of ecological processes affecting nestling body condition, sex-related mortality and sex ratio. We also found support for autumn natal dispersal as a functional mechanism to regulate the excess of one sex at the end of the parental care period. Since for logistical reasons we could not collect data at the brood-level, we cannot exclude the possibility that other facultative sex ratio adjustments occurred across groups (West 2009). Nonetheless, this study provides a novel example of the way facultative sex ratio adjustments can respond to biological features of the species (e.g. sexual dimorphism), environmental conditions (e.g. population-size) but also to non-facultative sex ratio changes (e.g. sex-differential mortality).

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Supplementary material (available online as Appendix oik.01889 at <[www.oikosjournal.org/readers/appendix](http://www.oikosjournal.org/readers/appendix)>). Appendix 1.