Article

Compensatory effect of egg size dimorphism on hatching asynchrony in Magellanic penguin

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Egg laying is one of the most important phases in a female bird's breeding cycle. Its cost is high because eggs contain all the resources needed for the development of an embryo. Variation in size and quality of eggs can have important long-term consequences for offspring survival. Hatching asynchrony is known to influence sibling competition in many bird species. Last-hatched chicks will have a competitive disadvantage throughout the pre-fledgling period because they are smaller. The aim of this study was to analyze the effect of hatching asynchrony and egg size variation on the growth and fledging success of Magellanic penguin *Spheniscus magellanicus* chicks after disentangling the effects of parental condition. We simultaneously manipulated egg size dimorphism, hatching asynchrony and parental condition by performing a cross-fostering experiment, creating broods with controlled egg size dimorphism and hatching asynchrony in a colony of Magellanic penguins located in Isla Quiroga, Santa Cruz, Argentina. We found that hatching asynchrony had a negative effect on last-hatched chicks, but this disadvantage was mitigated by egg size dimorphism in their favor. Moreover, females in good condition invest more in second than in first chicks, which, added to a greater investment by foster fathers, leading to offspring fledging in good condition. On the contrary, for the first-hatched chicks, we found that body condition of the biological father was an important factor for their growth. We conclude that raising more than one chick seems to be a decision based on parental condition throughout the breeding season.

Keywords: compensation of meternal effects, egg size dimorphism, hatching asynchrony, Magellanic penguin, parental body condition, seabird

Introduction

Egg laying is one of the most important phases in a female bird's breeding cycle. Its cost is high because eggs contain all the resources needed for the development of an embryo (Burley and Vadehra 1989). Variation in size and quality of eggs may have short-term effects, in growth and survival of chicks, lasting a few days after hatching (Reid and Boersma 1990, Christians 2002, Krist 2011, Barrionuevo and Frere 2014). This is possible because

high levels of hormones in eggs, like androgens, speed up embryonic development (Gil et al. 2003). However, egg size may also have important long-term consequences to offspring survival (Williams 1994, Carey 1996). Maternal effects (i.e. the influence of the maternal genotype or phenotype on the offspring phenotype (Wolf and Wade 2009)) can improve the success of a female's breeding performance by adjusting brood size according to current food conditions, because food availability is often unpredictable (Mock and Parker 1998). Females might adaptively modulate the asymmetries in competitive ability among siblings via egg size dimorphism and by differential deposition of resources within a clutch (Schwabl 1993, 1996, Müller et al. 2005, Barrionuevo and Frere 2014).

Hatching asynchrony (HA) (i.e. the days between the hatch of the first egg and the last egg) occurs when incubation starts before the last egg is laid (Magrath 1990, Stoleson and Beissinger 1995). Since chicks are fed from the moment they are born, a size hierarchy is generated within the nest, affecting the dynamics among siblings (Forbes and Mock 1994, Mock and Parker 1998). Thus, hatching asynchrony play a role in chick growth and survival (Hildebrandt and Schaub 2018). Boersma and Stokes (1995) found that size asymmetry influenced by hatching asynchrony, among other variables, affects the growth and survival of Magellanic penguin *Spheniscus magellanicus* chicks. Many hypotheses have been proposed to explain the occurrence of hatching asynchrony (Stoleson and Beissinger 1995). Hatching asynchrony may be understood within the context of the brood reduction hypothesis proposed by Lack (1947, 1954), according to which hatching asynchrony is an adaptation that facilitates mortality of the last-hatched chick by competition among siblings in years in which food resources are insufficient for rearing the whole brood. Thus, females may lay the number of eggs that could potentially be reared in years with good food resources (Mock 1984, Magrath 1990).

It has been proposed that variation in egg size and different allocation of yolk testosterone within clutches either mitigates or reinforces sibling competition through the hatchling size hierarchies (Howe 1978, Clark and Wilson 1981, Slagsvold et al. 1984, Muller and Groothuis 2012). For example, female of common canaries *Serinus canaria* vary the testosterone concentration of their eggs in a way that mitigates the effects of hatching asynchrony on the competitive abilities of last-hatched offspring (Schwabl 1993). Conversely, testosterone concentration decreases with laying order in the cattle egret *Bubulcus ibis* (Schwabl et al. 1997) a species in which the last hatching offspring are often subjected to high levels of sibling aggression, sometimes with fatal results (Ploger and Mock 1986). Regarding the intra-clutch egg size variation, Hébert and Barclay (1986) found that in the herring gull *Larus argentatus* last chicks from smaller eggs survived for a shorter time if left unfed compared with larger siblings. On the other hand, Whittingham et al. (2003) found that tree swallows *Tachycineta bicolor* that hatched from heavier eggs were larger and grew faster, during early stages in the nestling period.

In addition to the effect of hatching asynchrony and egg size dimorphism on initial hierarchies, food provisioning may limit chick growth and survival (Taylor and Roberts 1962, Ainley and Schlatter 1972, Boersma 1976, Cooper 1977). In agreement with this, the size asymmetry between siblings of Magellanic penguins is an important determinant of which chick is fed, since the largest chick receives more food (Blanco et al. 1996). Moreover, it has been shown that a chick's condition, and not its survival, is correlated with its parents' condition, especially that of the father (Barrionuevo et al. 2018). However, this last study could not disentangle whether the effect of parents was directly on chicks via food provisioning or indirectly via larger eggs when body condition was better (Cunningham and Russell 2000), which may also influence chick growth. So, there may be a confusion of genetics and environmental parental effects (Pelayo and Clark 2003, Velando et al. 2005).

Several cross-fostering studies have attempted to test the effects of hatching asynchrony and parental body condition, on the one hand and egg size dimorphism and parental condition, on the other (Reid and Boersma 1990, Bolton 1991, Amundsen et al. 1996, Hipfner and Gaston 1999, Giudici et al. 2017). However, none has evaluated the three variables together. The current paper presents the first experimental study on the importance of intra-clutch egg size dimorphism and hatching asynchrony, disentangled from parental condition. We determined the breeding performance of Magellanic penguins, controlling all three variables at the same time. To do so, we performed a cross-fostering experiment in which both egg size dimorphism and hatching asynchrony were manipulated in a Magellanic penguin colony in Isla Quiroga, Santa Cruz, Argentina. At this study site, females commonly produce a clutch size of two eggs, eggs hatch, on average, with a 1.8-day asynchrony (ranging from 0 to 4 days) (Barrionuevo 2015, Barrionuevo and Frere 2016) and egg size dimorphism (second egg volume–first egg volume) is 2% with the second egg larger than the first one (Barrionuevo 2015, Boersma and Rebstock 2010 for another colony). Thus, we hypothesize that: 1) egg size dimorphism in favor of the second egg provides extra help to the last-hatched chick, mitigating the disadvantage produced by hatching asynchrony, whereas hatching asynchrony favors the first-hatched chick since the largest chick receives more food (Blanco et al. 1996); 2) condition of foster parents is more important than condition of biological parents in growth and fledging success of both chicks. We expect that: 1) second chicks in asynchronous nests (hatching asynchrony other than 0) will grow and survive better as intra-clutch egg size dimorphism increases in favor of the second egg; 2) although females in better condition lay larger eggs (Barrionuevo and Frere 2014), it will be the foster parents' condition which will determine chick growth and fledging success.

Methods

Study area and species

Isla Quiroga, Santa Cruz, Argentina (47°45′S, 65°53′W) is an island situated 80 m from the coast, within Ria

Deseado (river inlet). It hosts a Magellanic penguin colony of around 1500 breeding pairs. Magellanic penguins have a seasonal breeding schedule. Males begin to arrive at the breeding colony in mid- to late September, and females in early October (Boersma et al. 2013). They usually lay two eggs (first egg = E1 and second egg = E2) in early October with 2% egg size dimorphism (E1-volume = 110.4 ± 0.42 cm^3; E2-volume = 112.7 ± 0.40 cm^3) (Barrionuevo and Frere 2014). The second egg is usually laid four days after the first one (Rebstock and Boersma 2011). Both parents share parental care (Boersma et al. 1990). Given that the temperature at which embryo development begins (26°C) is reached two days after the laying of the first egg because the brood patch begins to develop with the laying of the first egg (Barrionuevo and Frere 2012), chicks hatch in November, 1.8 days apart on average, with a natural variation of hatching asynchrony, ranging from −1 (the second egg hatches one day before the first) to four days (Boersma et al. 1990, Frere et al. 1998, Barrionuevo 2015). Chicks fledge at ~70 days of age, and suffer from facultative brood reduction, with the main cause of chick death in most colonies being starvation due to food scarcity (Boersma et al. 1990, 2015, Frere et al. 1998). However, the proportion of high/low quality prey in the diet is also important for nestling survival and growth (Barrionuevo et al. 2018). Chick diet is based on fish and squid (Frere et al. 1996). However, the proportion of these prey items are more similar to the father's diet than to the mother's since both parents consume the same prey but in different proportions (Ciancio et al. 2018); in fact, chick growth is more closely related to the father's condition than to mother's (Barrionuevo et al. 2018). At Isla Quiroga, Magellanic penguin breeding success (0.9 fledglings/nest-13-year study (Frere unpubl.)) is higher than in other colonies (0.53 fledgling/nest at Cabo Vírgenes (Frere 1998), 0.61 fledgling/nest at Punta Tombo (Boersma et al. 2013)).

Field methodology

In early October 2013, we randomly selected 150 nests without eggs, either with a male or a couple. We marked those nests with flagging tape with a unique nest number, and checked them every day before egg laying started. When an egg was laid, we marked the laying order (E1 or E2) and the nest number on the eggshell with a waterproof marker. We also measured the egg (maximum width and length) using a Vernier caliper (± 0.1 mm), from which we determined egg volume. Since the shapes of E1 and E2 differ, we used two different equations to determine volume of first- and second-laid eggs, as proposed by Boersma and Rebstock (2010) (volume of E1 = 1.6996 + (0.4967 × L × W2), volume of E2 = 8.2723 + (0.4758 × L × A2), where L and A are maximum length and width, respectively).

To assess parental body condition (BC), we captured females the day they laid the second egg, and males when they returned to the nest to take their first incubation shift.

We measured the following: weight (± 25 g; using a spring scale), length of foot (± 1 mm; using a ruler) from the bend in the tarsus to the end of the middle toenail, length of flipper (± 1 mm; using a ruler) from the joint between humerus and radius–ulna to the tip of the flipper, bill length (culmen, from the point where the upper mandible emerges from the forehead feathers to its tip; ± 0.1 mm) and bill depth (up to the nostrils; ± 0.1 mm) using a Vernier caliper. A body-size index was calculated as the first component extracted from a principal component analysis (PCA) of all the measurements (one index was calculated for males and another for females, since this species has a slight sexual dimorphism, being the males larger than the females (Gandini et al. 1992)). Residuals of body weight and size index regression were used as indices of body condition (Yorio et al. 2001).

Thirty-five days after the first egg was laid, we began to check nests daily to determine the timing of hatching of both eggs. For each clutch, we calculated hatching asynchrony (in days), weighed the chicks using a spring scale (± 1 g) and measured them (foot length, flipper length, bill length and bill depth, as measured in adults) with calipers (± 0.1 mm) on the day of hatching. After the second egg hatched, we weighed chicks every three days and measured them every six days. We used different spring scales according to chick weight (0–300 g, ± 2 g; 301–500 g, ± 5 g; 501–1000 g, ± 10 g; −1001 g, ± 25 g). After chicks were 24 days old, measurements were taken as for adults, and with the same accuracy. We considered chick body-size to be the scores of the principal component of a PCA of all measurements. We assumed a chick fledged if it was alive by 15 January (at 60 days old) and weighed at least 1900 g (Reid and Boersma 1990).

Cross-fostering experiment

On E1 laying day, we randomly categorized nests as either 'control nest' or 'manipulated nest'. If the nest had been categorized as 'control', we measured each egg (E1 and E2), marked it with the laying order and returned it to the nest. If the nest had been categorized as 'manipulated' when E1 was laid, we measured and marked the egg and replaced it with a plaster replica (the real E1 was safely placed in a plastic container inside the colony, under a bush sheltered from the sunlight but exposed to ambient temperature (Barrionuevo and Frere 2017)). The replica eggs were made of gypsum and were the same size and shape as penguin eggs, and were accepted by all females (Wagner et al. 2013, Barrionuevo 2015). For these nests, when E2 was laid, we measured the egg and added a second categorization to the nest: synchronous or asynchronous, according to the way it was manipulated, as follows:

a) If the nest was classified as synchronous, as soon as E2 was laid, we took it and placed it in another manipulated nest in which an E2 was laid on the same day. We also replaced the replica with a real E1 from another nest of origin. Thus, incubation of both E1 and E2 began on the same day (Fig. 1a–b).

b) If the nest was classified as asynchronous, we measured E2 at the time of laying and placed it in the plastic container. Simultaneously, we placed an E1 from another nest of origin in the nest, leaving the nest with a real E1 and an E2 replica. Two days later, we replaced the E2 replica with a real E2 from another nest of origin (Fig. 1c–d). Thus, incubation of E1 began two days before incubation of E2. The eggs that we placed in the manipulated nests (both synchronous and asynchronous) were chosen to generate clutches with different egg size dimorphisms. Thus, we produced synchronous and asynchronous nests either with E2s larger than E1s (Fig. 1a–c) or E1s larger than E2s (Fig. 1b–d). We also made sure that no egg was incubated in its nest of origin, or with a biological sibling. In all cases, the order of laying of each egg was respected (an E1 was replaced by another E1 and E2 was replaced by another E2).

Data analysis

To evaluate whether there was any effect derived from the manipulation, we ran two generalized linear mixed models (GLMM) with binomial distribution and logit link function. We used egg hatching success (whether or not the egg hatched) or fledging success (i.e. the chick was still alive on 15 January) as the response variable, and treatment (two-level factor = manipulated or control) as the predictor variable. Biological nest ID and foster nest ID, for control and treatment respectively, were used as a random variable to account for the lack of independence of the eggs or chick from the same nest.
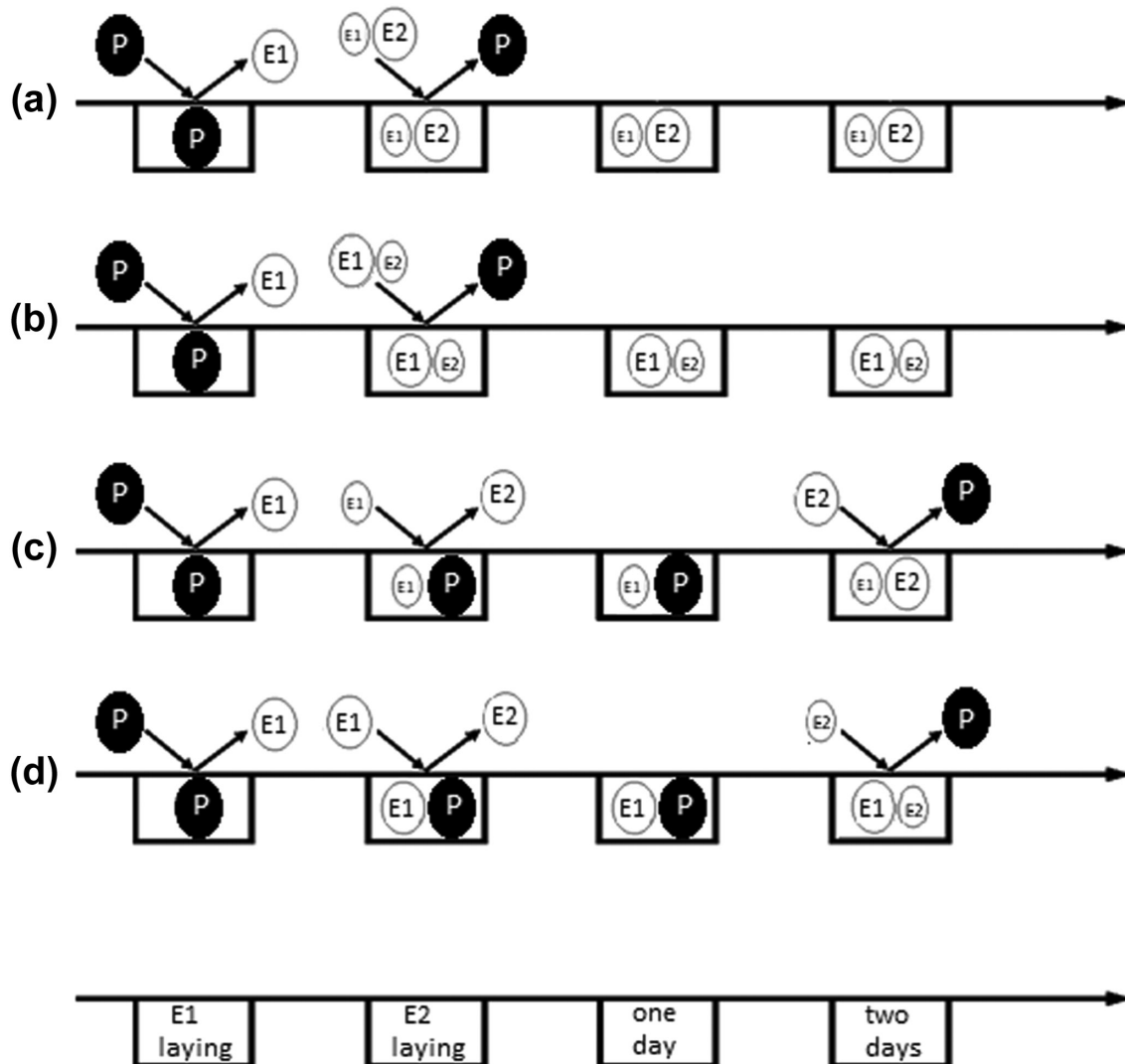

Figure 1. Illustration of the manipulation performed. For a and b: synchronous nests where (a) E2 was bigger than E1 and (b) E1 was bigger than E2. For c and d: asynchronous nests where (c) E1 was smaller than E2; and (d) nests where E1 was bigger than E2. P: plaster replica; E1: first egg laid; E2: second egg laid; E1 laying: day on which the first egg was laid; E2 laying: day on which the second egg was laid; one day: one day after the second egg was laid; two days: two days after the second egg was laid.

For the chick growth curves, we used a Gompertz growth curve for weight, and a sigmoidal growth curve for body size (Barrionuevo 2015); in both cases, curves had three parameters: asymptote value (a) (maximum weight [g]), rate of change (b) (growth rate [g day^{-1}]) and inflection point (c) (days until maximum rate [days]).

Effect of egg size dimorphism and hatching asynchrony on chicks

To assess the effect of egg size dimorphism and hatching asynchrony on the growth of the chicks, we ran four non-linear mixed models (NLMM), two for the first chicks and two for the second chicks. The response variables were growth in weight and body size index derived from the PCA made on all morphometric measures. The predictor variables were hatching asynchrony (which was entered in the model as a three-level factor: HA0, both E1 and E2 hatched on the same day; HA1, E2 hatched one day after E1; HA2, E2 hatched two days after E1), egg size dimorphism (which was entered in the model as a continuous variable) and the interaction between both. In all cases, the individual chick was the random variable. In these analyses, we only used nests with two fledged chicks.

To evaluate fledging success, we ran two generalized linear models (GLM), one for first chicks and one for second chicks, with a binomial distribution and log link function. The response variable was whether or not chicks fledged, and the predictor variables were hatching asynchrony, egg size dimorphism and the interaction between both. In this case, we only used nests with two hatched eggs.

Effect of parental body condition on chick performance

To evaluate chick growth dependence on parental condition, we developed four NLMMs, two for the first chicks and two for the second ones, each one with growth in weight and body size as response variables. The predictor variables were body condition index for the biological mother (BCBM), body condition index for the biological father (BCBF), body condition index for the foster mother (BCFM) and body condition index for the foster father (BCFF). In all cases, the chick was the random variable. In this case, we only used nests with two fledged chicks.

To assess the effect of parental condition on chick fledging success, we ran two generalized linear models (GLM), with a binomial distribution and log link function, one for first and another for second hatchlings. The response variable was whether or not chicks fledged, and the predictor variable was body condition of both the biological and foster parents. We only used nests with two hatched eggs.

All analyses were performed in the software R ver. 3.3.0 (<www.r-project.org>). For the linear models we used the 'lme4' package and for the non-linear models we used the 'nlme' package. When necessary, we tested for random effects by comparing the model with and without the random factor with a likelihood ratio test (Zuur et al. 2009). We then used a backwards selection procedure, removing the terms one by one in decreasing order of complexity (interactions first) and according to a decreasing value of P. After this, we compared models with and without the eliminated variable to the 'ANOVA' function and selected the model with the lowest AIC (Akaike information criterion) (Crawley 2012). For significant effects of the categorical variables, we performed multiple comparisons of the means with post hoc Tukey contrasts, using the 'glht' function of the 'multcomp' package within R.

Results

Our studied nests, with two eggs laid, were 114 (control = 32 nests, manipulated = 82 nests). Of the manipulated nests both eggs hatched in 62 nests (total: 76%, HA0: 71.4%, HA1: 80.8%, HA2: 75%). Out of the manipulated nests with two hatched eggs, both chicks survived as of 15 January, in 34 nests (total: 58.4%, HA = 0: 55.0%, HA1: 52.4%, HA2: 57.1%). Of the 28 remaining nests, in 14 nests at least one chick survived (total: 22.6%, HA0: 8.1%, HA1: 8.1%, HA2: 6.4%).

Methodological validation

Manipulation (egg swapping) had no significant effect on hatching success (control: n = 64 eggs, hatching success = 0.80 ± 0.16; manipulated: n = 164 eggs, hatching success = 0.85 ± 0.13; z = 0.867, p = 0.385) or on fledging success of chicks (control: n = 51 chicks, fledging success = 0.47 ± 0.25, manipulated: n = 140 chicks, fledging success = 0.58 ± 0.24; z = 1.375, p = 0.169).

Effect of egg size dimorphism and hatching asynchrony on chicks

The egg size dimorphism before manipulation (control nests + study nests prior to egg swap) at the colony was 2.15%, the second egg being larger than the first egg laid (volE1 = 111.6 ± 8.7 cm^3, volE2 = 114.1 ± 8.3 cm^3; t = −2.12, p = 0.035, n = 114 nests and 228 eggs). For manipulated nests, egg size dimorphism was similar to that of control nests (t = 0.23, df = 194, p = 0.817), dimorphism being 2.45% in favor of the second chick (volE1 = 111.1 ± 8.6 cm^3, volE2 = 113.8 ± 8.5 cm^3, t = −2.04, p = 0.043, n = 82 nests and 164 eggs). While average egg size dimorphism was similar for both control and manipulated eggs, size dimorphism had a larger range for manipulated eggs (range: Natural: Min = −15.23 cm^3, Max = 17.76 cm^3; Manipulated: Min = −20.86 cm^3, Max = 31.22 cm^3) (Fig. 2).

For first hatchlings (i.e. chicks that come from E1), neither increase in weight nor increase in body size were related to egg size dimorphism, hatching asynchrony or the interaction between them (Supporting information). For second

chicks, we found a significant negative relationship between model estimates for asymptotic weight and hatching asynchrony (L-ratio = 16.96, p < 0.001). Second chicks that had been born two days after their siblings achieved a lower estimate weight at fledging than those that had been born synchronously (HA0–2: Intercept value = 2924.05 g, estimate value = −298.35 g, t = −2.81, p = 0.005; HA1–2: Intercept value = 3120.05 g, estimate value = −494.36 g, t = −4.63, p < 0.0001). Conversely, we did not find any difference in synchronous nests compared to nests whose chicks hatch one day apart (i.e. hatching asynchrony of one day) (HA0–1: Intercept value = 2924.05 g, estimate value = 196.01 g, t = 1.8, p = 0.072). We found that egg size dimorphism, hatching asynchrony and the interaction between them did not affect growth in body size of second chicks (Supporting information).

Fledging success of the first chick was not related to egg size dimorphism, hatching asynchrony or the interaction between them (Supporting information), whereas for second chicks, fledging success was related to the interaction between egg size dimorphism and hatching asynchrony. Greater egg size dimorphism led to increased fledging success of second chicks that were born two days after their siblings as compared to those born the same day (Table 1, Fig. 3).

Table 1. To evaluate chick fledging success of second chicks of Magellanic penguins, we ran a general linear model, with fledging success as the response variable and egg size dimorphism (second egg volume–first egg volume), hatching asynchrony and the interaction between both as the predictor variable. All variables used are shown (n = 62 second chicks).

Response variable	Predictor variable	Estimate value	Z-value	p-value
Fledging success	Intercept	0.928	1.534	0.125
	Dimorphism	**−0.108**	**−2.116**	**0.034**
	HA0–1[1]	−0.372	0.483	0.629
	HA0–2[2]	−0.409	0.768	0.594
	Dim X HA0–1	0.091	1.477	0.139
	Dim X HA0–2	**0.152**	**2.457**	**0.014**
	Intercept	0.919	1.899	0.06
	HA1–2[3]	−0.079	−0.113	0.909
	Dim X HA1–2	−0.025	−0.537	0.592

[1]Hatching asynchrony between siblings in synchronous nests compared with one-day asynchrony.
[2]Hatching asynchrony between siblings in synchronous nests compared with two-day asynchrony.
[3]Hatching asynchrony between siblings with one-day versus two-day asynchrony.

Effect of parental body condition

For the following analyses, the correlation between body conditions within the couple was previously evaluated to discard collinearity between the two sexes, both for foster and biological parents. Correlation was not significant for physical condition of the biological and foster parents (Biological: r = −0.70, df = 32, p = 0.482; Foster: r = −0.43, df = 32, p = 0.670).

Regarding growth in weight of the first-hatched chicks, we found that estimate asymptotic weight was related to the body condition of the biological father (t = 2.07, p = 0.038), while the other variables were not significant (Supporting information). Females that mated with males in better body condition laid eggs whose chicks reached a greater estimate weight at the time of fledging (Intercept value = 3060.1 ± 54.7 g; BCBF: value = 225.3 ± 108.5 g). Regarding growth in body size, we did not find an effect of parental condition (Supporting information).

For second chicks, we found that growth rate in weight depended on the body condition of the foster father (t = 2.06, p = 0.042) (Supporting information). Foster fathers with higher body condition index raised chicks that grew faster than chicks raised by males with a poorer body condition index (Intercept value = 15.47 ± 0.32 g day^{-1}; BCFF: value = 1.34 ± 0.65 g day^{-1}). Furthermore, we found that estimate asymptotic body size, at the end of the rearing period, for second chicks depended on the body condition of both the foster father (t = 2.61, p = 0.016) and the biological mother (t = 2.64, p = 0.009) (Supporting information). Second chicks raised by foster fathers in better body condition reached a larger estimate asymptotic body size than those raised by males in poorer condition. Second chicks from eggs laid by females with better body condition also attained greater estimate body size than those from eggs
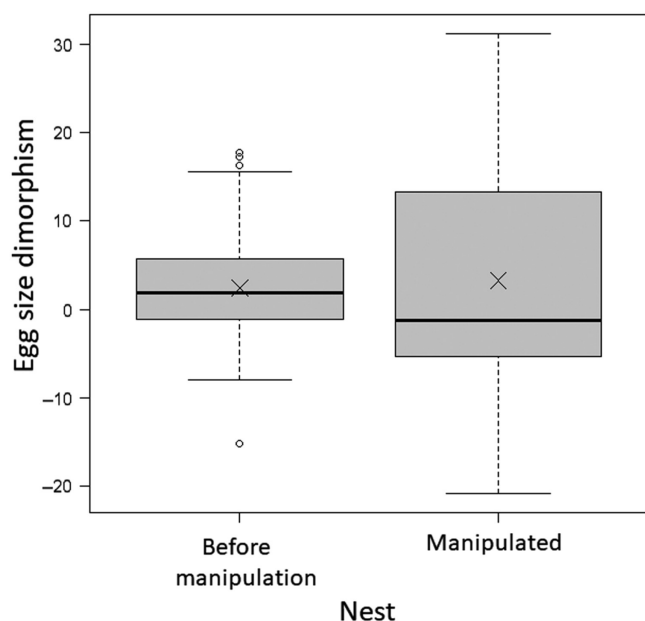

Figure 2. Differences between intra-clutch egg-size dimorphism (second egg volume–first egg volume) generated with manipulation and egg-size dimorphism before manipulation (i.e. control nest + study nest prior eggs swap) present in a Magellanic penguin colony (N$_{before}$ = 114 nest, N$_{manipulated}$ = 82 nest). The boxes indicate the 1st and 3rd quartiles, and the dark lines indicate the median. The means are indicated with crosses. Dotted lines indicate standard deviation. Points more than 1.5 times the inter-quartile range away from the box are shown with open circles.
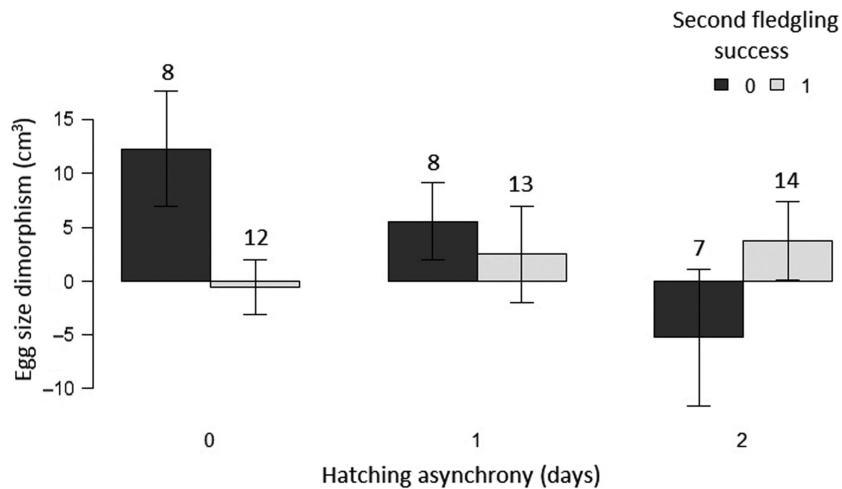
Figure 3. Differences in egg size dimorphism with the success of second fledglings for the three hatching asynchrony treatments for Magellanic penguin (n = 62 chicks). Dark bars show egg size dimorphism in clutches where second chicks did not fledge. Whereas light bars show nests where second chicks fledged successfully. The number above each bar represents the number of chicks per treatment. Standard errors are shown.

laid by females in poorer condition (Intercept value = 6.55 ± 0.07; BCFF: value = 0.38 ± 0.15; BCBF: value = 0.64 ± 0.24, t = 2.64, p = 0.009). Regarding fledging success, we found that it did not depend on the body condition of biological or foster parents (Supporting information).

Discussion

The results of this study suggest that maternal investment in size of second eggs can compensate for the disadvantage produced by hatching asynchrony on the second chicks of Magellanic penguins. Indeed, in two-day asynchronous clutches (i.e. nests whose chicks hatched two days apart), fledging success of second chicks was greater when egg size dimorphism (in favor of the second chick) increased. Additionally, hatching asynchrony had a negative effect on the growth of second chicks, with fledglings being lighter as hatching asynchrony increased. In addition, the asymptotic weight of first chicks depended only on the body condition of the biological father. On the other hand, for second chicks, the maternal effect influenced asymptotic body size. Moreover, body size increased and growth in weight was faster as the condition of the foster father improved (i.e. the body condition index was higher).

Methodological validation

This study found no difference between manipulated and control nests in terms of hatching success or fledging success of chicks, in agreement with previous studies on Magellanic penguins (Williams 1994, Barrionuevo and Frere 2014).

Hatching asynchrony and egg size dimorphism

This study found that E2 was 2.15% larger than E1, similar to the findings for the same colony by Barrionuevo and Frere

(2014), who reported a 2% egg size dimorphism in favor of E2. Nonetheless, egg size dimorphism seems to be a plastic trait, since at Punta Tombo colony (a colony of the same species located 650 km away), no egg size dimorphism was found and in the few years in which egg size dimorphism was found, it was in favor of the first egg laid (Boersma and Rebstock 2010). Furthermore, for our colony, it was found that females in better condition invested more in the last eggs than in the first eggs, when compared with females in worse condition (Barrionuevo and Frere 2014). When manipulating egg size dimorphism – generating some clutches with larger E1 and others with larger E2 – and hatching asynchrony, we found a compensatory effect of egg size dimorphism on hatching asynchrony in fledging success of the last hatchlings. This agrees with studies on passerines under the 'brood-survival strategy' hypothesis (Howe 1978, Slagsvold et al. 1984). A compensation with egg mass has been found in an altricial bird, but only during the early stages of chick development (Bitton et al. 2006). The opposite has been observed in seabirds in which maternal investment in eggs (hormones or egg size dimorphism) fails to compensate for hatching asynchrony, since Merkling et al. (2016) found that mothers seem to favor the competitiveness of their younger chick, in intermediate conditions of food availability, via egg yolk components, but the study also suggests that hatching asynchrony need to be small for efficient maternal compensation. In the cases where maternal effects compensate for hatching asynchrony, it is only during the early stages of growth (Reid and Boersma 1990). Agreeing with this, Braasch and Becker (2019) found that the influence of differential maternal investment across the laying sequence, such as egg size, on early growth and survival was subtle, gradually decreasing post hatching, and eventually was overridden by hatching asynchrony. The joint effect of egg size dimorphism and hatching asynchrony may not have been observed in previous studies (Rafferty et al. 2005, Barrionuevo and Frere 2014),

since the average egg size dimorphism was similar when comparing manipulated nests and natural nests, but the range for manipulated nest was greater than that for natural nests. Accordingly, in the present study, second chicks' growth was influenced by hatching asynchrony. Being born two days apart from its sibling generates a lower estimated asymptotic weight for second chicks, demonstrating a clear disadvantage, possibly because the older sibling obtained more food, as reported for Magellanic penguins in another colony (Blanco et al. 1996). The magnitude of hatching asynchrony is known to influence sibling competition in many bird species (Merkling et al. 2014). In Magellanic penguins, last-hatched chicks are at a competitive disadvantage throughout the pre-fledging period (Barrionuevo and Frere 2017). Here, we demonstrated that this disadvantage could be compensated by egg size dimorphism in favor of the last-hatched chick, agreeing with what was reported for altricial birds (Rosivall et al. 2005, Bitton et al. 2006).

Parental body condition

Female Magellanic penguins in good condition lay larger second eggs (i.e. second eggs are larger than first eggs) and this, better condition of females and larger eggs, are correlated to growth and survival of both first and second chick (Barrionuevo and Frere 2014). However, effects can be confounded, since the impact of the greater investment in second eggs may be masked by a greater investment during the chick-rearing period due to the better condition of the mother. To the best of our knowledge, our study is novel for seabirds, since it enables a better understanding of the effect of female investment in egg size disentangled from female investment during the chick rearing period. Contrary to predictions, the body condition of parents did not affect the two chicks equally. Body condition of the biological father was only important for first chicks. Barrionuevo and Frere (2014), working in the same colony, found that body condition of males was related to egg volume and yolk area, and that those variables affected chick growth and fledging success. However, this study did not include a cross-fostering experiment, as in our study. The relationship between biological males and the asymptotic weight of first chicks could either reflect an adjustment of investment in breeding by females according to the body condition of their partner (Cunningham and Russell 2000), or genetic traits transferred to the offspring (Andersson 1994). One possible explanation for the relationship between biological father and first chicks, but not the second chick, may be extra-pair copulations. Due to Magellanic penguin behavior during the laying period, second chicks are more likely to come from extra-pair copulations (Frere unpubl.) than first chicks. In this case, second chicks may not be related to the father, which the first chicks normally are.

Regarding the growth of the second chicks, we found that chick's asymptotic body size was influenced by body condition of the biological mother (indicating maternal effect on the egg). The breeding season considered in this study was 'intermediate' in terms of breeding success [0.94 chicks/nest (Marchisio 2018); average of 20 years for Isla Quiroga colony: 0.91 chicks/nest (Frere unpubl.)] and oceanic conditions (chlorophyll a and sea surface temperature) (Barrionuevo et al. 2018). Considering that the study year was 'intermediate', and that there was an increase in investment in second eggs by females in better condition (Barrionuevo and Frere 2014), it is possible that females in good condition invest more in last chicks to enhance their competitiveness via maternal effects, for instance, by egg size dimorphism (Barrionuevo and Frere 2014). Females may also contribute to the increased growth of their last chick by differential allocation of yolk substances (Schwabl 1993, Muller and Groothuis 2012, Benowitz-Fredericks et al. 2013, Merkling et al. 2016). As a result, we found a relationship between biological mother's condition and chick body size, even though they did not raise their biological chicks.

Furthermore, second chicks are also affected by the foster father's condition. Second chicks that were raised by foster fathers in better body condition had higher growth rates in weight and a higher estimated asymptotic body size than those raised by males in poorer body condition. This is similar to the strategy reported for procellariform species, in which the father feeds the chick with the lowest condition (Bolton 1995, Mauck and Grubb 1995, Weimerskirch et al. 1995, 2000, Hamer and Thompson 1997, Hamer et al. 1999), which may also be related to the higher food provisioning capabilities of males that is affected by the larger size of males compared to the size of females (Ciancio et al. 2018). This, in turn, is similar to what has been reported for other seabirds (Gray and Hamer 2001).

Conclusion

We corroborated the hypothesis that egg size dimorphism for Magellanic penguins gives second chicks a survival advantage, despite hatching asynchrony. On the other hand, growth of first chicks is only influenced by intrinsic egg factors, while growth of second chicks depends not only on hatching asynchrony and egg size dimorphism, but also on the maternal effect and the father's body condition. During an 'intermediate' year in terms of breeding success, raising a second chick seems to be a decision based on the condition of the parents throughout the breeding season. Females in good condition in the pre-laying period could invest more in second eggs than in first eggs, while foster fathers in good condition invest more in rearing of second chicks.

Author contributions

Nahuel Marchisio: Data curation (equal); Formal analysis (equal); Software (equal); Writing – original draft (equal). **Melina Barrionuevo**: Methodology (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal). **Esteban Frere**: Conceptualization (equal); Project administration (equal); Resources (equal); Writing – review and editing (equal).

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Data availability statement

Data are available from the Figshare Digital Repository: <https://doi.org/10.6084/m9.figshare.14798352.v1> (Marchisio et al. 2021).

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