Parental investment in eggs and its effect on nestling growth and survival in Magellanic Penguins

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Abstract. Life-history variables evolve in response to cost–benefit trade-offs. For birds, larger eggs are thought to be beneficial for development of offspring but are energetically costly to produce. Further, egg-size dimorphism within or between clutches can vary with proximate and ultimate causes. We undertook a correlational study to evaluate parental investment in eggs by Magellanic Penguins (*Spheniscus magellanicus*) and how it affects the growth and survival of nestlings in Puerto Deseado, Argentina, over 3 years. We evaluated the variables that affected egg-volume and yolk-area (using a non-destructive technique), and determined the effects of egg-volume and yolk-area on growth and survival of young. Females in good body-condition laid larger second eggs and, in good years (i.e. years of high reproductive success in the colonies of the study area), yolk-area of second eggs was larger than that of first eggs. We found a positive association between egg-volume and nestling body-size and yolk-area was positively related to nestling survival. Our results suggest that the size of eggs within clutches varied with year and female body-condition. Moreover we demonstrate for the first time that yolk-area is a strong predictor of nestling survival in Magellanic Penguins.

Introduction

In birds, the costs of reproduction are high (Gustafsson and Sutherland 1988; Hanssen *et al*. 2005) and this creates trade-offs that affect the evolution of life-history traits. Parental investment in reproduction is any action by parents that increases the fitness and survival of their offspring at the expense of some component of their own biological fitness, including their future mating success, fecundity or survival (Clutton-Brock 1991). The production and incubation of eggs and rearing of broods are behaviours that represent parental investment in reproduction.

Avian egg-production is an energetically expensive process (Monaghan *et al*. 1995, 1998; Monaghan and Nager 1997) and the variation in the total amount of resources allocated to a clutch, and the distribution of those resources within a clutch, can strongly influence maternal and offspring fitness (Bernardo 1996). Although the size of eggs is mostly heritable (Potti 1993), some authors suggest that females might allocate resources differentially to different eggs, increasing the fitness of the hatchlings (Styrsky *et al*. 2002; Hargitai *et al*. 2005; but see Whittingham *et al*. 2007). In this sense, variation in the size of eggs could act as an adaptive mechanism that may generate differences in egg-size with laying order enhancing or decreasing the survival of the last hatchling, and offset or accentuate the effects of hatching asynchrony on chick survival (Slagsvold *et al*. 1984). For example, hatching asynchrony could result in a competitive intra-clutch asymmetry between nestlings, leaving at a disadvantage the last-hatched nestling (Clark and Wilson 1981). Other authors suggest that the size of eggs is influenced primarily by proximate factors.

In passerines it has been shown that the body condition of a female (Styrsky *et al*. 2002; Ardia *et al*. 2006) or environmental factors during egg-formation, such as temperature (Hargitai *et al*. 2005) and availability of food (Hargitai *et al*. 2005; Ardia *et al*. 2006), affect egg-mass. Nilsson and Svensson (1993) concluded that energetic constraints during egg-formation are more important to intra-clutch variation than the adaptive response to a variable environment. In some bird orders, such as Sphenisciformes (penguins), environmental factors during the period of egg-formation should not influence the size of eggs because penguins use fat reserves for production of eggs (Meijer and Drent 1999). However, females are producing their eggs while migrating, and carry-over effects of the environmental and physiological constraints imposed by migration could affect egg-size dimorphism, as shown in Macaroni Penguins (*Eudyptes chrysolophus*) (Crossin *et al*. 2010).

In some bird species, larger eggs may result in higher hatching success (Perrins 1996) and lead to heavier hatchlings (Birkhead and Nettleship 1982; Rafferty *et al*. 2005; Whittingham *et al*. 2007), faster nestling growth-rates (Christians 2002; Whittingham *et al*. 2007) and long-term effects on the survival of offspring (Williams 1994; Carey 1996). However, yolk-size should also have an effect on weight of chicks and fledging success (Reid and Boersma 1990), because the yolk is the major source of energy and nutrients utilised by the developing embryo (Burley and Vadehra 1989; Deeming 2002). The yolk contains carotenoid pigments that act as antioxidants and regulate the immune function (Chew 1993), steroid hormones (Schwabl 1993), and

immunoglobulins (Graczyk *et al.* 1994), whereas the albumin is the main source of water and mineral ions for the embryo (Deeming 2002). Within species, most of the variation in egg-mass is a result of differences in the amount of water contained in albumin (Williams 1994), so egg-mass does not necessarily reflect yolk-mass (but see Ardia *et al.* 2006). The effects of a larger investment in egg-yolks on chicks and the variables that affect yolks are usually not studied, because, in order to assess yolk-size, the egg needs to be destroyed (but see Enemar and Arheimer 1989; Ardia *et al.* 2006).

There have been a number of studies of egg-volume in Magellanic Penguins (*Spheniscus magellanicus*). Boersma and Rebstock (2010) only found a significant difference between the mean volume of first and second eggs of a clutch in 5 of 24 years of study, whereas Rafferty *et al.* (2005) found that first eggs of a clutch were larger than the second ones, although they used a different equation for estimating egg-volumes. Boersma and Rebstock (2009*a*) also found that eggs in dimorphic clutches (clutches where eggs differed in volume) failed to hatch more often than eggs of normal (non-dimorphic) clutches, irrespective of the laying order, so that dimorphic clutches were considered to be energetically costly and non-adaptive. In addition, many studies have also analysed the effect of egg-volume on Magellanic Penguin chicks and found no relevant effects on weight of nestlings or fledging success (Reid and Boersma 1990; Rafferty *et al.* 2005; Boersma and Rebstock 2009*a*). Nonetheless, all these studies (Reid and Boersma 1990; Rafferty *et al.* 2005; Boersma and Rebstock 2009*a*) used an equation to determine egg-volume that Boersma and Rebstock (2010) showed to be inaccurate. None of the previous studies of Magellanic Penguin eggs analysed egg-content (i.e. yolk-area), which might better reflect parental investment in eggs and which might have a larger effect on nestlings than egg-volume *per se*.

The aim of our study was to test the hypothesis that proximate factors affect parental investment in eggs (egg-volume and yolk-area) of Magellanic Penguins. We also wanted to determine whether a larger investment in eggs might affect the weight, body-size and fledging success of chicks. Our study aimed to improve the knowledge of parental investment in eggs by using a reliable equation for estimating egg-volume (Boersma and Rebstock 2010). We also analysed egg-content using a non-invasive technique that allowed us to estimate yolk-area without destroying eggs and thus allowed us to evaluate the relationship between egg-content and growth of nestlings. Furthermore, mortality rates of eggs and chicks at Punta Tombo (a colony north of the colony we studied and where most previous studies have been conducted) are high – less than one in four eggs produce fledglings (Boersma *et al.* 1990; Boersma and Stokes 1995) and the population has been decreasing over the last 20 years (Boersma 2008). In contrast, our study area is notably different from other colonies (Schiavini *et al.* 2005) because breeding success is high (0.9 chicks per nest, 1991–2012) and the colony has been increasing over the last 20 years (E. Frere, unpubl. data). Thus, we expected different patterns of parental investment between our study colony and Punta Tombo given the different ecological conditions. We predicted (1) egg-volume and egg-yolks of second eggs would be smaller than those of first-laid eggs; (2) when the body-condition of parents is good, egg-volume and yolk-area would be larger; (3) year and laying date would affect

egg-volume and yolk-area; and (4) larger yolks and eggs would result in heavier and larger hatchlings.

Methods

Study species

The Magellanic Penguin lays two eggs, 4 days apart, between the beginning of October and mid-October; first eggs hatch after 41 days and second eggs after 39 days (Rebstock and Boersma 2011) with a 2-day asynchrony (range –1–4 days; Boersma *et al.* 1990). Incubation is shared approximately equally in alternating shifts by male and female parents, each performing an incubation shift of 15 days followed by a varying number of shorter shifts of 2–5 days duration until hatching (Boersma *et al.* 1990). Normally, females take the first incubation shift while males leave to forage before the second egg is laid (Boersma *et al.* 1990). Nestlings fledge at ~70 days old, and suffer facultative brood reduction (Lamey 1990).

Study site

We carried out the study at Isla Quiroga, an island only 80 m (at low tide) off the coast of Puerto Deseado, Santa Cruz Province, Argentina (47°45′S, 65°53′W), during three breeding seasons (October–January) 2010, 2011 and 2012. This island measures 600 m in length and ~98 m at the widest point, and although it is near the coast it still remains inaccessible for terrestrial predators. Approximately 1500 pairs of Magellanic Penguins breed on the island, with most nests beneath shrubs. The main predators of eggs and chicks are Kelp Gulls (*Larus dominicanus*) (E. Frere, pers. obs.).

Breeding parameters

We choose a random sample of 400 nests each year all across the island that we checked daily, beginning in early October, before laying started. From those nests we used 139 nests in 2010, 143 nests in 2011 and 187 nests in 2012. These nests were marked with flagging tape with a unique nest number. We omitted those nests that were used in previously years assuming that the couple returned to the same nest each breeding season (Boersma *et al.* 1990). In this way we avoided repeat measurements. All adults were identified with permanent metal tags (2×10 mm; National Band and Tag Company, Newport, KY) attached to the foot webbing with a unique identification number. When an egg was laid, we marked it with its order in the clutch (1 or 2) with a waterproof marker and measured it (maximum width and length) with Vernier callipers (± 0.1 mm; Mitutoyo, IL, USA), from which we determined egg-volume.

We used two different equations to determine volume of first- and second-laid eggs, as proposed by Boersma and Rebstock (2010), because the shape of first- (E1) and second-laid (E2) eggs differs:

$$\text{Volume}_{E1} = 1.699 + (0.497 \times \text{length} \times \text{width}^2)$$

$$\text{Volume}_{E2} = 8.272 + (0.476 \times \text{length} \times \text{width}^2)$$

To determine yolk-area, we took a standardised digital photograph of the eggs on the day of laying in an 'Ovolux' (Ardia *et al.* 2006; for diagram and dimensions, see Supplementary material, Fig. S1), which is a modified dark box with two

chambers. We did this only in 188 nests (2010, 38 nests; 2011, 58 nests; 2012, 92 nests). The bottom chamber has a round lamp with 24 LED bulbs (total 6 W and 90 lm W^{-1}) attached to the floor of the chamber and that projects light through a hole and tube that connects to the upper chamber. The egg is placed on the connecting tube in the centre of the upper chamber. The upper chamber has a cover with a central hole that accommodates the lens of the camera (Pentax, OPTIO E40, 8.1 MP, $3\times$ optical zoom, RICOH Imaging Company, CO, USA). When the LED is on, light passes through the egg without filtration, and we can take a clear picture of the yolk. After this, and with the use of the software Image Pro-Plus 4.5 (Media Cybernetics Manufacturing, Silver Spring, PA, USA), we measured the area of the yolk and total egg area in pixels, and then transformed this figure to megapixels (dividing by 1.048576×10^6). For all our analyses we assumed that yolk-area correlates with yolk-volume.

To assess parental body-condition, for all nests for which we marked and measured eggs, we captured females on the day of laying of the second egg and captured males when they returned to the nest to take on their first stint of incubation (~Day 15 of incubation). We measured the following: weight (±25 g; using a spring scale, Pesola AG, Baar, Switzerland); length of the foot (±1 mm; using a ruler), from the bend in the tarsus to the end of the middle toe nail; length of flipper (±1 mm), 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; using Vernier calliper) and bill-depth (up to the nostrils; ±0.1 mm). A body-size index was calculated as the first component extracted from a principal component analysis (PCA) of all measurements. Residuals of the weight $\times$ body-size index regression were used as indices of body-condition (Yorio *et al.* 2001). Indices of body-condition of males and females differs before or after a foraging trip. However, we wanted to compare body-condition within each sex not between sexes, so the differing timing of measurement of males and females does not affect such comparisons.

From 35 days after the laying of the first egg, 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 with a spring scale (±1 g; Pesola) on day of hatching, and measured them (foot-length, flipper-length, bill-length and bill-depth (as adult measurements)) with Vernier calipers (±0.1 mm). Some hatchlings (nestlings on the day they hatch) were fed before we arrived to measure them but others were not. We discarded measurements of those nestlings that weighed ≥100 g because those would likely have eaten. We weighed and measured chicks when they were 12, 24, 36 and 48 days old. We used different spring scales according to the nestlings weight ($0 > 300$ g, ±2 g; $301 > 500$ g, ±5 g; $501 > 1000$ g, ±10 g; >1001 g, ±25 g; all using Pesola scales). After chicks were 24 days old, measurements were done as for adults and to the same accuracy. We considered body-size of nestlings to be the principal component of a PCA of all measurements. We assumed that a chick had fledged if it reached 52 days old, weighed >1900 g and had begun moult.

Data analysis

We analysed the variables affecting egg-volume and yolk-area using two linear mixed models (LMM) with a Gaussian family

distribution and identity link function. We added nests as the random factor to the models to account for the lack of independence of eggs within a clutch, and egg-volume or yolk-area were response variables. We considered female and male body-condition to be predictor variables. We evaluated the effects of the following categorical variables: laying date (which was standardised across years), year (which was included as a 3-level factor: 2010, 2011, 2012), and laying order (which was entered in the model as a two-level factor: E1, first-laid egg; E2, second-laid egg). In addition, we analysed the effects of the following interactions: female body-condition and laying order; male body-condition and laying order; and year and laying order. The year is used as an estimator of marine environmental conditions and the availability of food in a breeding season, which can vary greatly between years (Frere *et al.* 1998; Boersma 2008; Boersma and Rebstock 2009*b*). We classified years as 'good years' or 'bad years' based on the median breeding success (0.9 fledglings per nest) of several colonies of Magellanic Penguins in the Puerto Deseado region over 11 years (E. Frere, unpubl. data): when success was higher than the median value, the year was classified 'good'; if lower or equal to the median value of success, the year was classified 'bad'.

To evaluate the effect of egg-volume and yolk-area on weight and body-size of hatchlings, we developed four LMMs with Gaussian family distribution and identity link function. We included nest as the random factor (as above) and hatchling weight or body-size as response variables. Predictor variables were year (2010, 2011, 2012), laying order (E1, E2; hatching order is the same as laying order) and yolk-area (yolk-model) or egg-volume (egg-model). Although Magellanic Penguins hatch asynchronically, and this generates a weight asymmetry between nestlings (Lamey 1990), we did not incorporate this into these models because it does not affect parameters on the day of hatching. We considered year and laying order in addition to egg-volume and yolk-area because we think they can influence growth of chicks and must be taken into account to achieve a reliable model. For this and the following analyses we included only nests in which both nestlings were alive.

To analyse the effect of egg-volume and yolk-area on growth of nestlings (weight and body-size), we ran 16 LMMs with Gaussian family distribution and identity link function. In each model, we used nest as the random factor (as above) and included the following as predictor variables: hatching asynchrony (as a 4-level factor: 0, 1, 2 and 3 days), year (2010, 2011, 2012), laying order (E1, E2), and yolk-area (yolk-models) or egg-volume (egg-models). The response variable was nestling weight at 12, 24, 36 or 48 days old, or body-size at 12, 24, 36 or 48 days old. For this set of analyses only, we omitted 2010 from the analyses that have yolk as a predictor variable (yolk-models) owing to the small sample size of yolk-area for that year.

We evaluated the role of egg-volume and yolk-area on fledging success with generalized linear mixed models (GLMM) with binomial distribution and a logit link function. We included fledging success as a dependent variable, nest as a random factor, and hatching asynchrony (0, 1, 2 and 3 days), hatching order (E1, E2), year (2010, 2011, 2012), and egg-volume (egg-model) or yolk-area (yolk-model) as predictor variables.

The sample sizes for the various statistical analyses described above varied greatly with stage of the nesting cycle

(egg, hatchling, nestling, fledgling). We determined egg-volume for a total of 469 nests and yolk-area for a total of 94 nests over the 3 years of the study. For analyses of nestling growth (at 12, 24, 36 and 48 days old), sample size was only 136 and 72 nests when we analysed the respective effect of egg-volume and yolk-area because analysis required both nestlings to be alive. Furthermore, sample size for such analyses decreased as nestlings grew older owing to nestling mortality.

In all analyses, 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 a decreasing order of complexity (interactions first) and according to a decreasing value of *P* (Crawley 2007). For these tests, we used the 'nlme' and 'lme4' packages in R version 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org, accessed 16 December 2010). For significant effects of the categorical variables, we performed multiple comparisons of the means with Tukey contrasts, using the 'glht' function of the 'multcomp' packages within R. For all other analyses we used Statistica version 7 (Statsoft Inc., Tulsa, OK). We report values as mean ± s.e. and considered differences to be significant at $P < 0.05$. All graphs were plotted using Sigma Plot 10.0 (Systat Software Inc., San Jose, CA, USA).

Results

Egg-volume and yolk-area

Mean egg-volume was 110.8 cm^3, ranging from 71.58 to 168.9 cm^3 (s.e. = 0.314 cm^3, n = 938 eggs). The mean yolk area was 1.07 megapixels, ranging from 0.81 to 1.37 megapixels (s.e. = 0.007 megapixels, n = 188 eggs). There was a significant correlation between yolk-area and egg-volume (simple regression, $R^2 = 0.529$, $P < 0.001$, n = 188 eggs) and yolk-area ranged from 65–89% of the total egg-area (average = 80%). There was also a significant relationship of the residuals of yolk-area regressed on egg-volume ($R^2 = 0.470$, $P < 0.001$).

Factors affecting egg-volume and yolk-area

For both LMMs analysing the variables affecting egg-volume and yolk-area (dependent variables), the effect of the random factor (i.e. nest) was significant (Tables 1, 2).

We found a significant relationship between egg-volume and body-condition of females and males, laying order and to the interaction between female body-condition and laying order (Table 1). Females paired with a male in good condition laid larger eggs than females paired with males in poor body-condition (Table 1). Further, for females in good condition, second eggs were larger than first eggs, whereas for females in poor condition, first eggs were larger than second eggs (Fig. 1, Table 1).

We also found a significant relationship between yolk-area and body-condition of females and males, laying order, year, and to the interaction between laying order and year (Table 2). Females in good condition laid eggs with larger yolks, and females paired with males in good condition also laid eggs with larger yolks (Table 2). The yolk-area of first and second eggs in 2012 was smaller than the yolk-area of first and second eggs in 2010 and 2011. In 2010, yolk-area of second eggs was larger

Table 1.   Linear mixed model of egg-volume of Magellanic Penguins against fixed and random effects

Only significant results are shown. n = 469 nests (938 eggs). E2, second egg laid. The significance of the random factor was tested with a likelihood ratio test

Fixed effects	Estimate	s.e.	*t*	*P*
Intercept	110.4	0.42	264.9	<0.001
Female body-condition	9.11	1.64	5.57	<0.001
Male body-condition	1.78	0.80	2.23	0.026
Laying order$_{E2}$	2.36	0.40	5.97	<0.001
Female body-condition × Laying order$_{E2}$	4.87	1.53	3.18	0.002
Random factor			χ^2	*P*
Nest			168.3	<0.0001

Table 2.   Linear mixed model of yolk-area of eggs of Magellanic Penguins against fixed and random effects

Only significant results are shown. n = 94 nests (188 eggs). E2, second egg laid. The significance of the random effects were tested with a likelihood ratio test

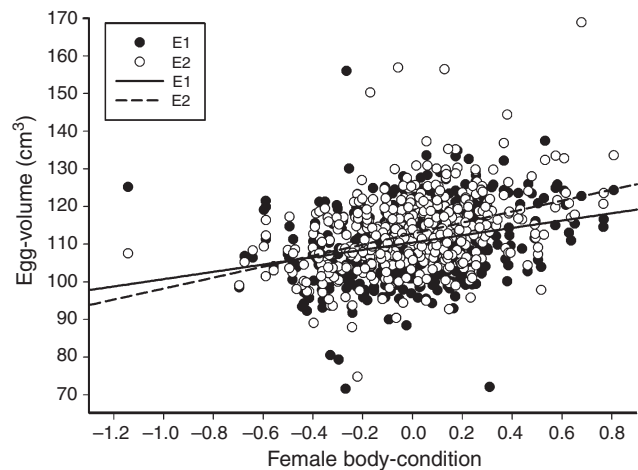| Fixed effects	Estimate	s.e.	*t*	*P*
Intercept	1.10	0.021	53.61	<0.001
Female body-condition	0.102	0.030	3.40	0.001
Male body-condition	0.032	0.016	2.00	0.049
Laying order$_{E2}$	0.067	0.019	3.40	0.001
Year$_{2011}$	−0.001	0.027	−0.05	0.950
Year$_{2012}$	−0.084	0.024	−3.41	0.001
Laying order$_{E2}$ × Year$_{2011}$	−0.054	0.025	−2.13	0.036
Laying order$_{E2}$ × Year$_{2012}$	−0.064	0.023	−2.75	0.007
Random factor			χ^2	*P*
Nest			30.597	<0.001

Fig. 1.   Relationship between female body-condition and egg-volume of first-laid (E1, black circles) and second-laid eggs (E2, white circles) of Magellanic Penguins (n = 469 clutches of two eggs (938 eggs)). Regressions are shown for first (solid line) and second eggs (short dashed line):   Volume$_{E1}$ = 110.4 + (9.719 × female   body-condition),   and Volume$_{E2}$ = 112.8 + (14.59 × female body-condition).

than that of first eggs; the pattern was the same in 2011 but the difference was not significant, whereas in 2012 there was no difference between yolk-area of first and second eggs (Fig. 2).

Females in good condition were paired with males in good condition (Spearman correlation, $R^2 = 0.187$, $P < 0.001$, $n = 466$ pairs). We found no differences in body-condition of adults between years (mean body-condition of females: 2010 = –0.048, 2011 = 0.047, 2012 = 0.043; one-way analysis of variance (ANOVA), $F_{2,468} = 0.553$, $P = 0.575$, $n = 469$ females; mean body-condition of males: 2010 = –0.098, 2011 = 0.016, 2012 = 0.037; one-way ANOVA, $F_{2,465} = 0.007$, $P = 0.994$, $n = 466$ males).

Effects of egg-volume and yolk-area on chicks

Hatchlings

Mean weight of first-hatched young (from first-laid egg) was 78.4 ± 0.75 g (range 40–98 g, $n = 345$) and that of second-hatched young was 78.2 ± 0.77 g (range 44–98 g, $n = 331$). Hatching asynchrony was 1.21 ± 0.14 days ($n = 469$ nests). Reproductive success was higher in this study than that reported for other colonies of the region (Schiavini *et al.* 2005) for each of the three the years of the study (2010, 1.16 fledglings per nest; 2011, 1.14; 2012, 0.64).

We found a significant and positive relationship between weight of hatchlings (i.e. on day of hatching) and egg-volume (L-ratio = 148.6, $P < 0.001$, $n = 284$ nests; Fig. 3*a*) and weight of hatchlings and yolk-area (L-ratio = 6.36, $P = 0.012$, $n = 74$ nests; Fig. 3*b*) but only egg-volume was a significant predictor of body-size of hatchlings (L-ratio = 33.27, $P < 0.01$, $n = 284$ nests). Weight of hatchlings and year were also positively related (egg-model: L-ratio = 111.6, $P < 0.001$, $n = 284$ nests; yolk-model: L-ratio = 11.43, $P = 0.003$, $n = 74$ nests) but body-size of hatchlings and year were not; hatchlings from 2012 were
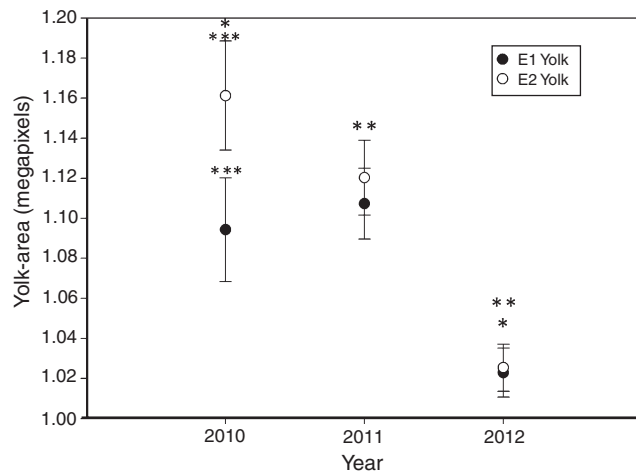
lighter than those from 2010 and 2011. There was also a significant relationship between laying order and body-size in the yolk-area model only (L-ratio = 4.55, $P = 0.033$, $n = 60$ nests), with second hatchlings larger than first hatchlings. The random effects of all these models were significant ($P < 0.001$).

Growth of nestlings

There was a significant and positive relationship between egg-volume and chick weight up until 12 days old, and to body-size up until 48 days old (Table 3). There was, however, no significant relationship between yolk-area and weight or body-size of nestlings (Table 3, but see 24-day-old nestlings). The remaining variables also showed significant relationships: year had a significant effect on weight and body size of nestlings throughout the period of growth; hatching order had a significant effect on weight of nestlings but not body size; and hatching
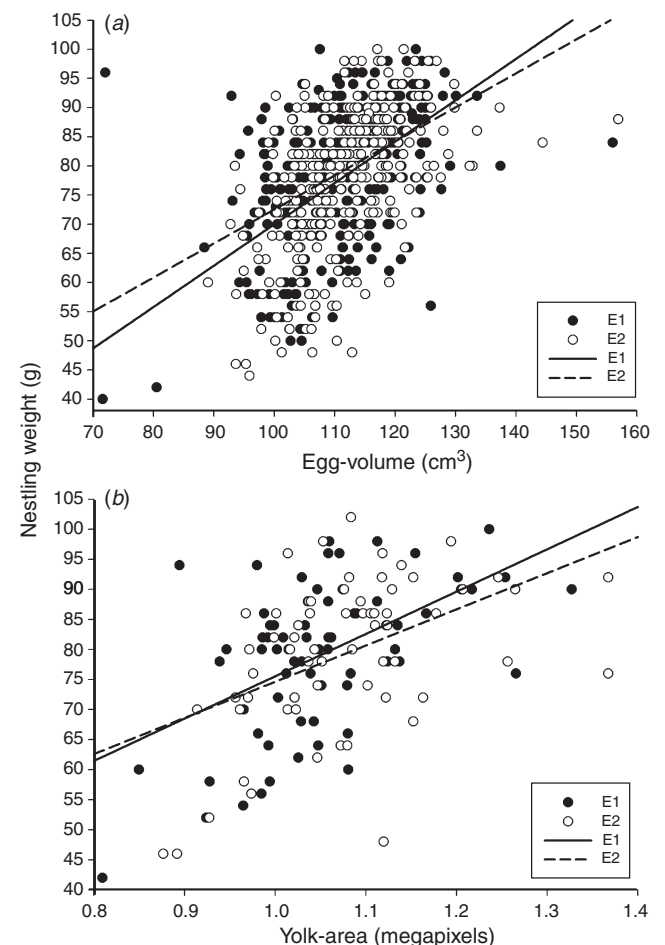

Fig. 2. Mean yolk-area (±s.e.) of first and second eggs (E1, black circles; E2, white circles; $n = 94$ clutches of two (188 eggs)) of Magellanic Penguins for each year of the study (2010, 2011, 2012). Significant results of Tukey contrasts are: *, differences between E1$_{2010}$ *v.* E1$_{2012}$, E1$_{2010}$ *v.* E2$_{2012}$, E2$_{2010}$ *v.* E1$_{2012}$ and E2$_{2010}$ *v.* E2$_{2012}$ (all $P < 0.011$); **, differences between E1$_{2011}$ *v.* E1$_{2012}$, E1$_{2011}$ *v.* E2$_{2012}$, E2$_{2011}$ *v.* E1$_{2012}$ and E2$_{2011}$ *v.* E2$_{2012}$ (all $P < 0.001$); ***, differences between E1$_{2010}$ *v.* E2$_{2010}$ ($P = 0.008$).

Fig. 3. Relationship between weight of nestling Magellanic Penguins (at day = 0) and: (*a*) egg-volume ($n = 469$ nests, 938 eggs) and (*b*) yolk-area ($n = 94$ nests, 188 eggs). First eggs (E1), black circles; second eggs (E2), white circles. Linear regressions graphed for E1 (solid lines) and E2 (dashed lines). Regression equations: Nestling weight *v.* egg-volume: Weight$_{Nestling1}$ = 14.17 + (0.584 × Volume$_{E1}$); Weight$_{Nestling2}$ = –0.976 + (0.709 × Volume$_{E2}$); Nestling weight *v.* yolk-area: Weight$_{Nestling1}$ = 5.096 + (70.44 × Yolk-area$_{E1}$); Weight$_{Nestling2}$ = 14.42 + (60.21 × Yolk-area$_{E2}$).

Table 3. Factors affecting weight and body-size of Magellanic Penguin chicks

Separate linear mixed models were run for chicks at 12, 24, 36 and 48 days old. Egg-models are used as a predictor variable of the egg-volume and yolk-models are used as a predictor variable of the yolk-area

Nestling age	Weight		Body-size index	
Model	F	P	F	P
Variables				
12 days old				
Egg-model (n = 136 nests)				
Hatching asynchrony	8.54	0.04	2.16	0.54
Hatching order	6.79	0.01	1.84	0.16
Year	17.74	<0.0001	0.72	0.70
Egg-volume	19.20	<0.0001	9.08	0.004
Yolk-model (n = 72 nests)				
Hatching ay?chrony	7.64	0.05	0.57	0.90
Hatching order	4.90	0.03	0.58	0.45
Year	19.81	<0.0001	0.24	0.62
Yolk-area	0.22	0.64	0.95	0.33
24 days old				
Egg-model (n = 118 nests)				
Hatching asynchrony	4.167	0.244	15.624	<0.0001
Hatching order	2.206	0.137	1.714	0.191
Year	51.993	<0.0001	17.492	<0.0001
Egg-volume	2.423	0.119	4.264	0.039
Yolk-model (n = 56 nests)				
Hatching asynchrony	2.477	0.479	8.235	0.041
Hatching order	11.809	<0.0001	3.461	0.064
Year	35.327	<0.0001	9.647	0.002
Yolk-area	2.477	0.479	8.235	0.041
36 days old				
Egg-model (n = 102 nests)				
Hatching asynchrony	2.168	0.441	0.139	0.708
Hatching order	1.578	0.665	9.455	0.024
Year	5.845	0.015	0.547	0.459
Egg-volume	34.770	<0.0001	49.147	<0.0001
Yolk-model (n = 46 nests)				
Hatching asynchrony	3.279	0.351	6.124	0.106
Hatching order	19.063	<0.0001	0.669	0.414
Year	37.562	<0.0001	32.405	<0.0001
Yolk-area	0.760	0.383	0.388	0.533
48 days old				
Egg-model (n = 98 nests)				
Hatching asynchrony	3.137	0.371	4.056	0.255
Hatching order	3.730	0.053	1.293	0.255
Year	44.161	<0.0001	39.562	<0.0001
Egg-volume	0.181	0.671	3.704	0.054
Yolk-model (n = 42 nests)				
Hatching asynchrony	0.957	0.811	1.473	0.688
Hatching order	9.045	0.003	1.039	0.308
Year	42.811	<0.0001	31.601	<0.0001
Yolk-area	0.144	0.704	0.169	0.681

Table 4. Generalised linear mixed model with binomial distribution of survival of nestling Magellanic Penguins

Two models were run, with egg-volume (egg-model) or yolk-area (yolk-model) as predictor variable

	χ^2	P
Egg-model (n = 136 nests)		
Egg-volume	2.23	0.135
Hatching asynchrony	1.27	0.735
Year	16.42	0.001
Hatching order	2.04	0.153
Yolk-model (n = 72 nests)		
Yolk-area	12.93	0.012
Hatching asynchrony	5.86	0.119
Year	2.77	0.251
Hatching order	12.65	0.013

also important in determining fledging success; the proportion of first chicks that fledged was larger than that of second chicks (Table 4).

Discussion

Our results demonstrate that parental body-condition affects egg-volume and yolk-area, and that egg-volume dimorphism within a nest can change according to the body-condition of the female and that yolk-area dimorphism can change with year. We also show that yolk-area is a strong predictor of nestling survival, and that egg-volume affects weight of nestlings at early ages (<12 days old) and body-size of nestlings from hatching until 48 days old. Previous studies on Magellanic Penguins analysed the adaptive value of the intra-clutch dimorphism in egg-volume and its effect on fledging success and survival of chicks (Reid and Boersma 1990; Rafferty *et al.* 2005; Boersma and Rebstock 2009*a*). However, the method of determining egg-volume in these studies was inaccurate (Boersma and Rebstock 2010) and examined only the total volume of the egg and not characters of the yolk. We determined accurately and analysed both egg-volume and yolk-area as important indicators of parental investment in breeding. Both variables were correlated, but only 53% of the variance in yolk-area was attributed to egg-volume. Moreover, we demonstrate how different factors can affect the different components of the egg. These results showed the importance of studying both egg-volume and yolk-area in conjunction as measures of parental investment because each component of the egg is produced at different times and is influenced by different factors. Had we analysed only egg-volume, for example, we would have overlooked the effect of year on the parental investment in eggs, and the influence of this component of parental investment on fledging success.

At our study site, females in good condition produced larger eggs with larger yolks. The body-condition of females can explain variation in egg-mass in many passerine birds (Christians 2002; Hargitai *et al.* 2005; Ardia *et al.* 2006). In passerines, females are normally captured during incubation to measure their condition. In a study of Magellanic Penguins breeding at Punta Tombo, females were captured at the point of incubation as in our study, and their weight positively related to total volume of a clutch (Boersma and Rebstock 2009*a*). However, no relation between female body-condition and egg-mass has been found in other

asynchrony affected body-size only at one age: 24 days old (Table 3).

Success

There was a significant positive relationship between fledging success and yolk-area but not with egg-volume: chicks that fledged successfully were from eggs with larger yolks (Table 4). Other factors, such as hatching order and year, were

species such as Imperial Cormorants (*Phalacrocorax atriceps*), although female Cormorants in that study were captured later in the nesting cycle than in our study, between the middle of the incubation and early chick-rearing periods (Svagelj and Quintana 2011). Furthermore, in our study we found that body-condition of males was also significantly related to variation in egg-volume and yolk-area. This positive relationship could either reflect adjustment of investment in breeding by females according to the body-condition of their partner (Cunningham and Russell 2000) or females in good condition selecting mates in good condition. We found that parental body-condition within a nest was positively related. Our results indicate that egg-volume and yolk-area are plastic traits in Magellanic Penguins at this colony, and vary in relation to body-condition of the female and male.

Another proximate factor we analysed was each year of the study, and which was found to have a significant relationship with yolk-area but not egg-volume. Rafferty *et al.* (2005) found that intra-clutch variation in egg-volume of Magellanic Penguins did not differ significantly between years, indicating that allocation of resources between eggs by females was fairly consistent from year to year. We found, however, differences in yolk-area within nests and in total yolk-area between years. Apparently, in penguins, egg-volume but not yolk-area is consistent between years. It appears that the yolk-content, which is the source of nutrients for the embryo, is more plastic than total egg-volume between years.

We also found that egg-volume and yolk-area varied with laying order. If we pool data across all 3 years, second eggs were larger than first eggs, contrary to the original prediction of our study and to results of other studies of Magellanic Penguins (Rafferty *et al.* 2005; but see Boersma and Rebstock 2010). This variation in egg-volume within nests is novel for the genus and is consistent with a brood-survival hypothesis, which is a common pattern in small species of birds (Mead and Morton 1985; Whittingham *et al.* 2007). This hypothesis proposes that females invest more in last-laid eggs to mitigate the disadvantage of these last-hatched chicks when competing for food with the earlier-hatched chicks (Slagsvold *et al.* 1984; Budden and Beissinger 2005). We do not think this hypothesis fits our breeding system because it predicts equal survival of nestlings in a brood, contrary to our results. Nevertheless, one possible explanation for the variation in egg-size is that it could be advantageous to invest more in the second egg in our colony because it has a high probability of survival depending on the year. The reproductive success during the years of the study was high, so Magellanic Penguins are more likely to raise two chicks in Puerto Deseado when compared to those in Punta Tombo. Although second chicks were more likely to starve, in two of the three years of this study (2010 and 2011), 67% of the nests with two hatched eggs fledged both nestlings (M. Barrionuevo and E. Frere, unpubl. data). Further, if the first hatchling is not fed before the second egg hatches, which is only 1 day in our colony, the asymmetry resulting from this asynchrony in hatching might be compensated for by a larger second egg. Moreover, variation in egg-volume and yolk-area within nests was related to female condition and year. In this sense, one possible explanation for intra-clutch variation in egg-size is that if females are in good condition, or if the year is predicted to be a good year, they could afford to raise both chicks by investing more in the second egg, particularly given the

features of the study colony: high fledging success, high survival of second eggs, and reduced periods of hatching asynchrony. Conversely, variation in egg-volume within clutches could be a consequence of the environmental conditions that females experience while producing eggs (Galbraith 1988; Järvinen 1991), which can place energetic and nutritional constraints on females. For example, availability of food (Nilsson and Svensson 1993) and ambient temperature (Magrath 1992) influence egg-size in some species of bird. If availability of food varies during the period of egg-formation it could result in intra-clutch variation in the size of eggs (Perrins 1970). Although it has been shown in another penguin species (Adelie Penguin, *Pygoscelis adeliae*) that they produce their eggs based on fat reserves, environmental conditions while accumulating those reserves are likely to have some influence on the eggs later produced by the female. Marine environmental conditions could also indirectly affect egg-volume (Monaghan *et al.* 1998) and there may also be carry-over effects of migration from non-breeding grounds to their breeding colonies (see below). Crossin *et al.* (2010) found that environmental and physiological constraints during migration are important determinants of variation in egg-size in female Macaroni Penguins because egg-formation occurs during migration. It is not known at what point during their migration that female Magellanic Penguins produce eggs (i.e. whether they are close to or far from colonies). Magellanic Penguins migrate from their non-breeding range, as far as south of Brazil, to their breeding colonies, located south of Argentina (Williams 1995). Magellanic Penguins that cover more distance when migrating to their breeding colonies from their non-breeding range might be more affected by migratory carry-over, and this may partly explain why we found intra-clutch differences in egg-size whereas such differences were not observed in northern colonies, like Punta Tombo. Further studies tracking Penguins during migration and comparing multiple colonies at different distances from their non-breeding range are needed.

Some avian studies have found that egg-mass had an effect on growth of chicks for only a few days after hatching (Krist *et al.* 2004), whereas other studies have found the effect lasted longer (Birkhead and Nettleship 1982) and even affected chick survival (Risch and Rohwer 2000). Reid and Boersma (1990) found that egg-volume in Magellanic Penguins (independent of parental quality) influenced weight of chicks only during the first 10 days after hatching. We found a similar effect of egg-volume on chick weight up until 12 days after hatching, but egg-volume determined body-size of chicks right up until fledging. However, no relationship was found between growth of nestlings and yolk-area, which only significantly influenced nestling weight on the day of hatching. We also found, like Rafferty *et al.* (2005), no significant relationship between egg-volume and fledging success but, remarkably, greater fledging success of chicks hatching from eggs with larger yolks. We did not expect that either egg-volume or yolk-area would influence fledging success because the nestling stage of penguins is long and other factors should influence survival more (Lamey 1992; Williams *et al.* 1993; Rafferty *et al.* 2005). Therefore, because egg-volume affects nestling growth through an effect on body-size, yolk-area might be influencing nestling survival through another pathway other than weight or size of chicks, because we found no effect of yolk-area on these parameters. Crossin *et al.* (2012) has shown

that female albatrosses with low levels of yolk precursors produce chicks that fail to fledge. It is possible the levels of hormones or antibodies deposited in the yolk could be important influences on chick survival (Rubolini *et al.* 2005), so that yolk-composition, related to yolk-area, might be determinants of fledging success. However, these results could also be a consequence of females and males in good condition laying eggs with larger yolks and being better able to raise the chicks until they fledge. Cross-fostering experiments are needed to help disentangle the effects of parental body-condition from any egg-effects. Therefore, if fledging success was merely a consequence of parents' body-condition, and yolk-area did not matter, in 2012, when fledging success was at its lowest, parents should have had, during this breeding season, the lowest body condition. However, in 2012 parental body-condition was not the lowest recorded in our study site.

To conclude, we found that maternal investment in eggs was greater for second eggs when the females were in good condition and in the year with the highest breeding success. The historical population parameters of this colony indicate that a high percentage of second eggs survive and that the period of hatching asynchrony is reduced, which could make it advantageous to invest more in second eggs. Nevertheless, migratory carry-over effects should be taken into account because it has been shown to influence within-egg-size variation in other penguins. The results of our study highlight the importance of cross-fostering experiments in future studies to understand fully the causes of variation in egg-size in Magellanic Penguins. Moreover, beyond egg-volume and its relation to body-size of chicks, yolks are crucial to fledging success as yolk-area affected nestling survival. Our results show that under varying environmental conditions (between years and colonies), patterns of parental investment in eggs by Magellanic Penguins also vary greatly.

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