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Extreme intra-clutch egg size dimorphism is not coupled with corresponding differences in antioxidant capacity and stable isotopes between eggs

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ABSTRACT

Oviparous females need to allocate resources optimally to their eggs in order to maximize their fitness. Among these resources, dietary antioxidants, acquired by females and transferred to the eggs during egg formation, can greatly affect the development and survival of the embryo and chick. In crested penguins, incubation starts after the second and last egg is laid and, as opposed to many other bird species, this egg hatches first, thereby enhancing the survival of the chick. Here, we assessed whether antioxidant and isotopic composition could underlie these differences between eggs within clutches of southern rockhopper penguins (*Eudyptes chrysocome chrysocome*). The second-laid egg had higher total antioxidant capacity than the first-laid egg, although this was not due to higher antioxidant concentration but to its higher mass. This suggests that resources are allocated by females at a constant rate in both eggs within clutches. Accordingly, we found a strong correlation for isotopic compositions between eggs suggesting that resources were allocated similarly to each egg within the clutch. Overall, we found little evidence for a significant role of antioxidant and isotopic compositions to explain differences in terms of embryo/chick development between eggs in crested penguins. However, since our results suggest a constant rate of antioxidant transfer from females to eggs, limiting the mass of the first-laid egg might represent a strategy for females to spare antioxidant defences and preserve self-maintenance.

1. Introduction

By varying the transfer of antioxidant resources to their eggs (Royle et al., 2003), female birds can strongly affect the development and survival of their offspring (Wilson, 1997; Møller et al., 2008; Deeming and Pike, 2013). However, as these antioxidant resources are of dietary origin and are therefore limited (Royle et al., 2003), their transfer to the eggs may be costly and deleterious to laying females in terms of self-maintenance (energy limitation hypothesis; Roff, 1992; Stearns, 1992; Zera and Harshman, 2001). Depositing antioxidants into eggs may indeed decrease the females' own antioxidant defences and increase their levels of oxidative damage (Morales et al., 2008; Giordano et al., 2015). This is likely to explain why females feeding in habitats with low food availability, or having a poor nutrient status, a poor body condition, or low antioxidant defences decrease the allocation of antioxidants (as well as other nutrients) to their eggs (Hargitai et al., 2006; Navara et al., 2006; Isaksson et al., 2008). Consequently, laying females may have to modulate the total antioxidant transfer to their clutch and/or its distribution within this clutch in relation to their own condition. This suggests the existence of an interplay between female feeding behaviour, transfer of resources to their eggs, their own antioxidant status, and that of their eggs.

Stable isotope analysis can provide insights on the effects of variation in resource availability on antioxidant allocation. The isotopic composition of an animal's tissue is indeed directly linked to that of its diet. Isotope ratios may vary spatially and reflect the habitat in which animals feed, in addition nitrogen isotope ratios generally increase from prey to consumer tissue and may reflect trophic levels (see Cherel et al., 2007 and references therein). Stable isotope analysis therefore represents an indirect method to assess foraging strategies, which can be used to determine the relative importance of feeding sources (Gauthier et al., 2003). For instance, penguins predominantly feeding on krill (with low $\delta^{13}C$ and $\delta^{15}N$ values) show low isotope values compared to penguins predominantly feeding on fish (with high $\delta^{13}C$ and $\delta^{15}N$ values) (Polito et al., 2011; Dehnhard et al., 2016). Krill also contains higher levels of lipophilic antioxidants (mostly astaxanthin) than fish (Tou et al., 2007). Therefore, markers of antioxidant capacity

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and isotopic composition can correlate in seabirds' blood and eggs (Hipfner et al., 2010; García-Tarrasón et al., 2014; Beaulieu et al., 2015).

Crested penguins (genus *Eudyptes*) present a unique extreme intra-clutch egg size dimorphism, with the first-laid egg (the A-egg) being 55–75% the size of the second-laid egg (the B-egg) (Warham, 1975; Demongin et al., 2010). In the typical two-egg nests of southern rockhopper penguins (*Eudyptes chrysocome chrysocome*), the hatching success is similar between A- and B-eggs, but A-eggs take longer to hatch and produce smaller chicks that usually do not survive due to almost obligatory brood reduction (St. Clair, 1996; Poisbleau et al., 2008). This disadvantage of A-eggs relative to B-eggs in terms of embryonic development and chick survival becomes apparent only when both eggs are incubated together, but not if A-eggs are incubated alone. In contrast, embryonic development and chick survival remain constant in B-eggs irrespective of incubation conditions (Poisbleau et al., 2008). This suggests that A-eggs are more sensitive to incubation conditions than B-eggs. Differences in the composition of both eggs may contribute to these differences in sensitivity, and low levels of antioxidant defences in A-eggs may be related to a high sensitivity to incubation conditions resulting in a slow(er) embryonic development. For instance, interspecific comparisons in birds have shown that the speed of embryonic development increases in relation to the quantity of maternal antioxidants transferred into the egg (Deeming and Pike, 2013). If this pattern holds true also at the intra-clutch scale, we could expect A-eggs to have a lower antioxidant capacity than B-eggs in rockhopper penguins, which might contribute to their slower development (Prediction 1).

Southern rockhopper penguins are considered as typical capital breeders (Jönsson, 1997; Meijer and Drent, 1999) with females acquiring body reserves before and during migration to breeding sites, fasting and relying solely on body reserves during egg production. Under these assumptions, we predict that the oxidative status and isotopic compositions of females should be directly reflected in the oxidative status and the isotopic compositions of their eggs (Prediction 2).

Nevertheless, we observed that females return to the colony about 18 days before clutch initiation (Poisbleau et al., 2015) while egg production lasts about 23 days, with A-egg production starting four days prior to B-eggs (Grau, 1982; Crossin et al., 2010). We therefore assume that females are still likely to acquire food for direct (at least A-) egg production while migrating. The relative contribution of the exogenous (acquired at the time of egg production during migration) and endogenous (acquired away from the breeding site) nutrients into eggs should therefore be reflected by a difference in egg isotopic composition (see Hobson et al., 2015; Ramírez et al., 2015 for examples of studies using this effective and precise method for tracing nutrient allocation into eggs). Under these conditions, we expect a difference in isotopic composition between A- and B-eggs if both endogenous and exogenous nutrients are used for clutch production (with a higher contribution of exogenous resources in A-eggs), but no difference in isotopic composition between eggs if only endogenous reserves are mobilised (Prediction 3).

Finally, we expect females limited in their ability to invest in reproduction (because of a poor nutritional state) to lay more dimorphic clutches, thus favouring the survival of at least one (the B-) egg/chick. We therefore expected a relationship between female condition (body mass, oxidative status or isotopic composition) and intra-clutch differences in terms of egg quality (mass, antioxidant status and isotopic composition; Prediction 4).

2. Materials and methods

2.1. Study site and birds

The study was carried out during the austral summer 2012 on southern rockhopper penguins breeding at the "Settlement Colony" (51°43′S, 61°17′W) on New Island, Falkland/Malvinas Islands. The breeding biology of this population that held about 8300 pairs in 2012 has been described previously in Poisbleau et al. (2008). The birds mainly breed in open rocky areas fringed by tussac grass *Poa flabellata*. Males arrive in the colony first (early October) and establish nest sites. Females arrive a few days later, for pairing and copulation in late October/early November. Egg laying is very synchronised within this population, taking place in less than two weeks (see Poisbleau et al., 2008).

Since 2006, we have gradually marked 461 randomly-chosen adult females in the colony, equipping them with 23-mm long glass-encapsulated electronic transponders (TIRIS, Texas Instruments, USA). We determined the sex of birds through bill measurements within pairs, with males typically having larger bills than females (Poisbleau et al., 2010).

2.2. Adult manipulation

During the 2012 laying period, we visited the study site daily to follow egg laying. We randomly chose 40 marked females, which were homogeneously distributed within the study site and the laying period. They were captured on the day they laid their A-egg (*i.e.* date of laying onset). After covering their head with a hood to minimize stress, we collected up to one ml of blood from the brachial vein, using a 23-gauge needle and heparinized syringe. Blood samples, which were collected within three minutes after capture, were stored on ice while still in the colony, and were centrifuged within three hours. Red blood cells and plasma samples were stored at −20 °C in separated 1.5-ml Eppendorf tubes until analysis.

We weighed each female to the nearest 20 g with an electronic balance following Poisbleau et al. (2010). Since indices of body condition may not be more precise than body mass itself (Schamber et al., 2009), we did not control for structural size in further analyses.

Table 1
Within-clutch comparison of egg composition traits between A- and B-eggs. Mean mass (in g), antioxidant capacities (OXY, in $mmol^{-1}$ HOCl neutralised), total antioxidant capacities (Total OXY, in $mmol^{-1}$ HOCl neutralised), stable carbon isotope ratios ($\delta^{13}C$, in ‰) and nitrogen isotope ratios ($\delta^{15}N$, in ‰) in the yolk, albumen and whole egg are compared within clutches between A- and B-eggs using paired *t*-tests and paired correlations.

	Mean ± SE		Paired correlation		Paired *t*-test	
Trait	A-egg	B-egg	r	*P*-value	t	*P*-value
Yolk mass	19.4 ± 0.5	22.0 ± 0.3	**0.643**	**<0.001**	**7.357**	**<0.001**
Albumen mass	64.1 ± 1.1	81.1 ± 1.2	**0.839**	**<0.001**	**26.29**	**<0.001**
Egg mass	95.8 ± 1.6	118.4 ± 1.4	**0.834**	**<0.001**	**25.74**	**<0.001**
OXY_{Yolk}	2726 ± 73	2705 ± 90	−0.184	0.256	−0.169	0.866
$OXY_{Albumen}$	349 ± 6	349 ± 6	0.140	0.388	−0.008	0.994
Total OXY_{Yolk}	$26.10^6 \pm 10^6$	$30.10^6 \pm 10^6$	0.182	0.261	**2.497**	**0.017**
Total $OXY_{Albumen}$	$11.10^6 \pm 0.3.10^6$	$14.10^6 \pm 0.3.10^6$	**0.466**	**0.002**	**10.23**	**<0.001**
Total OXY_{Egg}	$37.10^6 \pm 10^6$	$44.10^6 \pm 10^6$	0.212	0.188	**4.260**	**<0.001**
$\delta^{13}C_{Yolk}$	−19.4 ± 0.1	−19.4 ± 0.1	**0.912**	**<0.001**	−0.134	0.894
$\delta^{13}C_{Albumen}$	−19.6 ± 0.1	−19.4 ± 0.1	**0.993**	**<0.001**	**15.01**	**<0.001**
$\delta^{15}N_{Yolk}$	14.1 ± 0.1	14.3 ± 0.2	**0.927**	**<0.001**	**2.998**	**0.005**
$\delta^{15}N_{Albumen}$	15.3 ± 0.2	15.4 ± 0.2	**0.985**	**<0.001**	**3.571**	**0.001**