

# Egg-laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*

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**In birds, experimentally increased egg production can reduce maternal condition, parenting ability and survival, and the quality of the eggs themselves. Such costs probably reflect resource limitation, but the identity of the resource(s) in question remains unclear. Carotenoids are antioxidants and immunomodulators that birds can only obtain in their diet. Trade-offs in the allocation of limiting carotenoids between somatic maintenance and egg production could therefore be an important factor underlying reproductive costs. We show that in wild lesser black-backed gulls, *Larus fuscus*, dietary carotenoid availability (i) constrained the capacity to re-lay following clutch removal; and (ii) affected the relationship between yolk mass and egg mass. However, whether carotenoids are limiting for egg production directly, by stimulating the synthesis or antioxidant protection of yolk precursors, or indirectly via effects on maternal health, requires further study.**

**Keywords:** antioxidants; life-history trade-offs; lipid peroxidation; oestrogen

## 1. INTRODUCTION

The high costs of egg production are a major factor underlying life-history diversity in birds, influencing chick-rearing capacity (Monaghan *et al.* 1998), susceptibility to parasitism (Oppliger *et al.* 1996) and survival (Visser & Lessells 2001), while egg quality itself diminishes with increasing egg number (Bernardo 1996; Nager *et al.* 2000). Experiments have suggested that such costs reflect nutritional constraints: clutch enlargement through egg removal reduces maternal pectoral-muscle protein condition (Monaghan *et al.* 1998), and dietary supplementation can increase egg production (e.g. Clifford & Anderson 2001). However, previous supplemental feeding studies have manipulated supplies of whole foods such as egg itself (but see Ramsay & Houston 1998), which contain many putatively limiting nutrients (e.g. amino acids, lipids, antioxidants). The role of specific nutrients, parti-

cularly micronutrients, in shaping the costs of egg production remains unclear (Williams 1994; Bernardo 1996).

One hypothetically limiting class of micronutrients for avian egg production is carotenoid pigments (Royle *et al.* 1999; Blount *et al.* 2000). Carotenoids are antioxidants and immunomodulators that birds cannot synthesize *de novo* and must obtain in their diet (Olson & Owens 1998; Møller *et al.* 2000). Maternally derived carotenoids in egg yolk are thought to enhance egg quality (Haq *et al.* 1996; Blount *et al.* 2002a,b). Supplemental feeding experiments have recently shown that carotenoid availability can limit antioxidant and immune defences in adult birds (Blount *et al.* 2002b, 2003), but whether egg-laying capacity is also carotenoid-limited remains to be seen. We have previously shown in wild lesser black-backed gulls (*Larus fuscus*) that carotenoid supplementation does not influence the proportion of females that lay, or the timing, size or mass of first clutches (Blount *et al.* 2002a,b). However, since gulls use endogenous nutrient reserves for egg production (Houston *et al.* 1983; Bolton *et al.* 1993; Monaghan *et al.* 1998), critical levels of carotenoid accumulation could pre-date their arrival at the breeding grounds. Therefore, we tested the capacity of carotenoid-supplemented and control females to replace a lost clutch. Clutch loss through predation is common (Cramp 1983), therefore individuals often must face the decision of whether to re-lay within a short time-scale.

## 2. METHODS

The experiment was carried out between April and August 2000 at a large gull colony (*ca.* 24 000 pairs) on Walney Island, Cumbria, UK. On 9 April, about one month before laying started, we randomly allocated nests in the centre of the colony to receive either a control-diet ( $n = 25$ ) or carotenoid-diet supplement ( $n = 25$ ). Birds in both treatments received a 20 g pellet of solid vegetable fat (Van den Bergh Foods Ltd, Crawley, UK) each night at the nest, which was fortified in the carotenoid-diet group with 2 mg of carotenoids. This supplement comprised 40 mg of Oro Glo Layer (active ingredients 1.8% lutein and 0.2% zeaxanthin by mass; Kemin Europa NV, Herentals, Belgium), 5 mg of Carophyll Red (10% canthaxanthin by mass; La Roche, Basel, Switzerland) and 7 mg of Rovimix (10%  $\beta$ -carotene by mass; La Roche), mixed in a ratio similar to the profile of carotenoids occurring naturally in the eggs of this species (Blount *et al.* 2002a). Supplemental feeding continued until 4 days after laying of replacement clutches began, which typically is the day of completion of a modal-sized clutch of three. Birds that failed to re-lay were supplementally fed until 20 days after removal of the first clutch (no birds laid after that interval).

All of the nests were visited daily and fresh eggs were collected and replaced with dummies. Consistent with our earlier findings (Blount *et al.* 2002a,b), carotenoid supplementation did not significantly affect the date of first laying (control-diet,  $10.44 \pm 6.26$  May (mean  $\pm$  s.e.); carotenoid-diet,  $10.80 \pm 6.80$  May;  $t$ -test,  $t_{48} = 0.20$ ,  $p = 0.85$ ), clutch size (control-diet, 3(3,3) eggs (median (first and third quartiles)); carotenoid-diet, 3(3,3) eggs; Mann-Whitney  $U$ -test:  $U = 275.50$ ,  $p = 0.28$ ) or mass (control-diet,  $229.67 \pm 6.35$  g; carotenoid-diet,  $212.46 \pm 9.74$  g; ANCOVA controlling for clutch size: maternal diet,  $F_{1,46} = 0.22$ ,  $p = 0.64$ ; clutch size,  $F_{1,46} = 120.35$ ,  $p < 0.0001$ ; interaction, NS). Four days after laying commenced (i.e. on the typical day of clutch completion, see above), we captured females at the nest using walk-in traps, recorded body mass using a spring balance ( $\pm 2.5$  g) and tarsus length using a sliding calliper ( $\pm 0.1$  mm), then collected 0.5 ml of blood from the tarsal vein into a heparinized syringe. All females then received an intraperitoneal injection of 1.5 ml of physiological saline before being released at the nest. This injection, which should have no adverse effect on females, provided an experimental control as part of a different study that will be reported elsewhere. Blood was kept at  $4-6$  °C for up to 4 h before plasma was collected by centrifugation, and then stored at  $-20$  °C until analysis of total carotenoids by HPLC, as described by Blount *et al.* (2003). All females recommenced incubation on the same day that they had been captured. The following morning, we removed dummy clutches to induce re-laying. Replacement A- (i.e. first-laid), B- and C-egg mass was recorded ( $\pm 0.1$  g) using an electronic balance, and then B-egg yolk was separated from albumen and

weighed ( $\pm 0.1$  g). (Replacement A- and C-eggs were not sampled destructively because they were used in another experiment that will be reported elsewhere).

Statistical models were developed using backward elimination starting with the highest-order interaction. Two-tailed  $\alpha = 5\%$ . Values are means  $\pm 1$  s.e.

### 3. RESULTS

The latency to re-lay was not affected by treatment (control-diet,  $11.22 \pm 0.37$  days; carotenoid-diet,  $11.75 \pm 0.34$  days; ANCOVA controlling for replacement clutch mass: maternal diet,  $F_{1,38} = 0.00$ ,  $p = 0.95$ ; replacement clutch mass,  $F_{1,38} = 6.51$ ,  $p = 0.015$ ; interaction, NS). However, one-third more carotenoid-diet females re-laid than controls (figure 1a). In an analysis based on control-diet birds only, whether a female re-laid was significantly related to her plasma carotenoid levels (non re-layers,  $2.66 \pm 1.52 \mu\text{g ml}^{-1}$ ; re-layers,  $7.15 \pm 1.19 \mu\text{g ml}^{-1}$ ) but not her body condition (logistic regression: plasma carotenoids,  $\chi^2 = 5.72$ , d.f. = 1,  $p = 0.017$ ; body mass,  $\chi^2 = 1.17$ , d.f. = 1,  $p = 0.28$ ; tarsus length,  $\chi^2 = 0.60$ , d.f. = 1,  $p = 0.44$ ; all interactions, NS).

Carotenoid supplementation did not influence the mass of replacement clutches (ANCOVA controlling for clutch size: maternal diet,  $F_{1,38} = 0.19$ ,  $p = 0.67$ ; clutch size,  $F_{1,38} = 145.45$ ,  $p < 0.0001$ ; interaction, NS; no difference in clutch size between treatments: Mann-Whitney  $U$ -test:  $U = 188.00$ ,  $p = 0.32$ ). However, whereas in the control-diet group larger eggs had disproportionately small yolks, in the carotenoid-diet group yolk and egg mass covaried proportionately (figure 1b).

### 4. DISCUSSION

Carotenoid supplementation increased the capacity of gulls to replace a lost clutch of eggs: one-third more carotenoid-diet females re-laid compared with controls. One possible explanation is that supplemental carotenoids increased the synthesis and/or antioxidant protection of yolk precursors. Carotenoids and retinoic acid (a vitamin A metabolite, ultimately derived from pro-vitamin A carotenoids such as  $\beta$ -carotene) stimulate expression of oestrogenic enzymes, which have been shown to increase oestrogen production *in vitro* (Ng *et al.* 2000; Hughes *et al.* 2001). Oestrogen stimulates hepatic expression of the genes that code for synthesis of very low-density lipoprotein (VLDL) and vitellogenin, the main sources of yolk lipid and protein, respectively (Speake *et al.* 1998). We have previously shown in gulls that maternally derived carotenoids increase levels of antioxidant activity (Blount *et al.* 2002b) and reduce susceptibility to peroxidation in the egg yolk (Blount *et al.* 2002a). VLDL has a highly unsaturated lipid profile (Speake *et al.* 1998), and consequently is vulnerable to peroxidation (Blount *et al.* 2000). Therefore, carotenoid supplementation could have increased the production and/or antioxidant protection of circulating vitellogenin and VLDL, giving rise to an increased capacity to produce eggs.

If carotenoids enhance the capacity to lay, why then did not carotenoid-diet females lay larger replacement eggs? Carotenoid supplementation clearly influenced replacement B-egg composition: yolk mass and egg mass covaried proportionately in the carotenoid-diet group, whereas in the control-diet group yolk mass declined with increasing

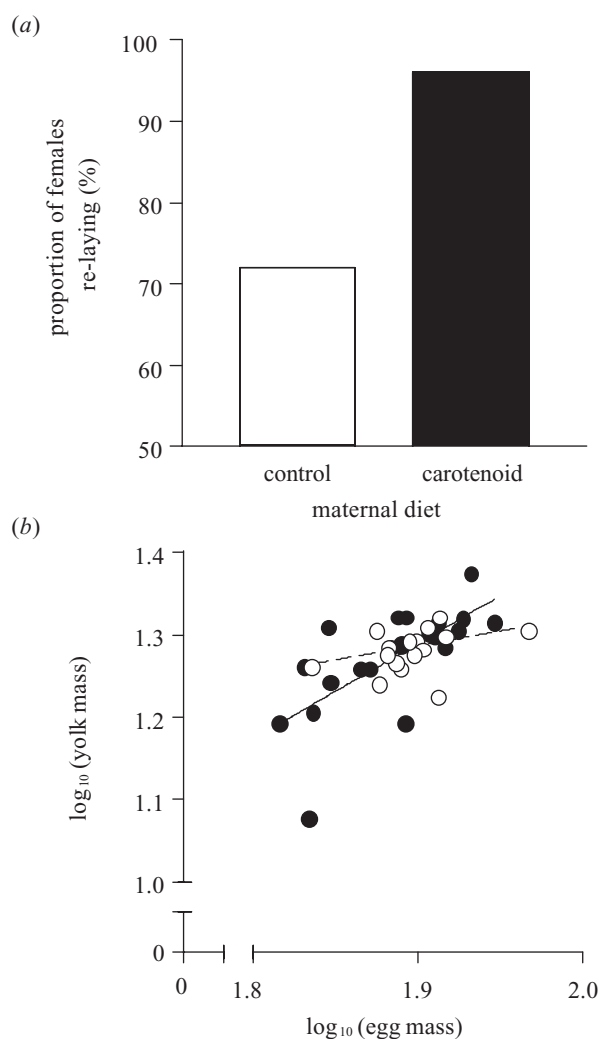


Figure 1. Egg production in relation to maternal diet (control-diet, open bar/open circles and broken line; carotenoid-diet, filled bar/filled circles and solid line); (a) A larger proportion of carotenoid-diet females re-laid following clutch removal than controls. Logistic regression: maternal diet,  $\chi^2 = 6.81$ , d.f. = 1,  $p = 0.009$ ; laying date,  $\chi^2 = 0.35$ , d.f. = 1,  $p = 0.55$ ; interaction, NS. (b) The relationship between yolk mass and egg mass differed between treatments (log : log ANCOVA with  $\log_{10}(\text{B-yolk mass})$  as the dependent variable: maternal diet,  $F_{1,35} = 3.95$ ,  $p = 0.06$ ;  $\log_{10}(\text{B-egg mass})$ ,  $F_{1,35} = 8.67$ ,  $p = 0.006$ ; laying date,  $F_{1,35} = 0.43$ ,  $p = 0.52$ ; maternal diet by  $\log_{10}(\text{B-egg mass})$  interaction,  $F_{1,35} = 3.97$ ,  $p = 0.05$ ; all other interactions, NS), covarying proportionately in the carotenoid-diet group ( $1.16 \pm 0.26$  [slope  $\pm$  s.e.],  $t(\text{slope} = 1) = 0.61$ , d.f. = 22,  $p = 0.55$ ) but allometrically in the control-diet group ( $0.35 \pm 0.24$ ,  $t(\text{slope} = 1) = 2.77$ , d.f. = 14,  $p = 0.015$ ). Omission of the carotenoid-diet egg with the lightest yolk from the analysis did not significantly change the  $\log_{10}(\text{B-yolk mass}) : \log_{10}(\text{B-egg mass})$  relationship ( $0.88 \pm 0.20$ ;  $t(\text{slope} = 1) = 0.61$ , d.f. = 21,  $p = 0.55$ ; not significantly different from the full dataset:  $F_{1,43} = 0.72$ ,  $p = 0.40$ ).

egg mass. Decreasing yolk size with increasing egg size is typical in seabirds, so variation in egg size largely reflects changes in albumen (i.e. protein and water) content (Williams 1994). Possibly, therefore, some carotenoid-diet re-layers had relatively low supplies of such macronutrients compared with the control-diet re-layers. This stands to reason because, under natural feeding conditions,

carotenoids can only be consumed with macronutrients—indeed, tissue carotenoids are often bound to lipids or proteins (Olson & Owens 1998)—so maternal levels of carotenoids and macronutrients should covary. Interestingly, in gulls it is believed that maternal body condition, particularly the pectoral-muscle protein condition, influences her egg-laying capacity (Houston *et al.* 1983; Monaghan *et al.* 1998). This is thought to reflect the importance of protein for deposition into eggs and maintenance of the egg-laying machinery, and also maintenance of pectoral muscle condition that affects the ability of females to forage (Houston *et al.* 1983; Monaghan *et al.* 1998; Nager *et al.* 2000). However, our analysis of clutch replacement in relation to maternal body condition and plasma carotenoid levels in control-diet females suggested that the latter was a stronger determinant.

We cannot exclude some alternative explanations. If carotenoids enhanced the efficiency of immune defences this could have resulted in an increased availability of other limiting nutrients; running the immune system is costly in terms of protein and energy turnover (Lochmiller & Deerenberg 2000). It is also conceivable that a healthier individual should have better foraging efficiency. These explanations predict that sustained carotenoid supplementation should result in increased maternal body condition, and possibly larger eggs (see previous paragraph), but we have not found such effects (Blount *et al.* 2002b; this study). However, to understand better the relative importance of carotenoids and other nutrients such as protein for egg production requires manipulation of the availability of a specific nutrient while controlling for others.

In conclusion, this study of gulls has shown that egg-laying capacity is carotenoid-limited. Trade-offs in carotenoid allocation between somatic maintenance and egg production might therefore underlie the costs of avian egg production (see § 1). Our results also underline the possibility that maternal expression of carotenoid-based integument pigmentation could reveal foraging efficiency (as in males; e.g. Hill 1992) and egg-laying capacity (Blount *et al.* 2000; Amundsen & Forsgren 2001). However, the particular mechanism by which carotenoid availability determines egg-laying capacity is not known and requires further study.

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