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Pre-fledgling oxidative damage predicts recruitment in a long-lived bird

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Empirical evidence has shown that stressful conditions experienced during development may exert long-term negative effects on life-history traits. Although it has been suggested that oxidative stress has long-term effects, little is known about delayed consequences of oxidative stress experienced early in life in fitness-related traits. Here, we tested whether oxidative stress during development has long-term effects on a life-history trait directly related to fitness in three colonies of European shags *Phalacrocorax aristotelis*. Our results revealed that recruitment probability decreased with oxidative damage during the nestling period; oxidative damage, in turn, was related to the level of antioxidant capacity. Our results suggest a link between oxidative stress during development and survival to adulthood, a key element of population dynamics.

Keywords: oxidative damage; nestling stage; antioxidants; fitness; recruitment; European shag

1. INTRODUCTION

Empirical evidence has shown that stressful conditions experienced during early development can influence physiological and life-history traits during later life (reviewed in [1]), such as survival to adulthood [2], body condition at sexual maturity [3] and thereby fitness in animals including humans [3–5]. However, our knowledge of long-lasting consequences of stressful development remains limited, particularly in wild animals.

Oxidative stress, the imbalance between production of reactive oxygen species (ROS) and antioxidant defences leading to oxidative damages, is a potentially important physiological cost implicated in life-history trade-offs [6] and senescence [7]. During early development, organisms are prone to experience high levels of oxidative stress [8] and stressful conditions in very early life may alter the balance between ROS production and antioxidant defences and repair capacity [9,10]. In vertebrates, young may experience a high metabolic activity during rapid growth that in turn causes oxidative damage to the organism [11,12]. It has been proposed that oxidative damage during development may potentially constrain organisms' fitness in adulthood [13]. Nevertheless, evidence for negative associations between oxidative damage during early life and subsequent performance in wild animals is scarce.

Here, we tested whether recruitment probability was related to pre-fledging oxidative damage in a long-lived seabird, the European shag *Phalacrocorax aristotelis*. The European shag is a large altricial bird. Shag nestlings exhibit high growth and metabolic rates [14,15], and hence they presumably experience high levels of oxidative stress. In this species, early conditions influence telomere erosion, and it has been suggested that oxidative stress is the proximal cause of this relationship [16]. In three breeding colonies of shags, we first examined whether oxidative damage is related to circulatory antioxidant levels in full-grown nestlings. Then, using extensive monitoring data of marked birds during a four-year period, we examined if oxidative damage of shag nestlings influences their recruitment probability.

2. MATERIAL AND METHODS

This study was carried out in three breeding colonies of the European shag (Illas Cíes, Illa de Ons and Sagres) at the Parque Nacional das Illas Atlánticas (Galicia, Spain; see the electronic supplementary material). Between April and June 2006, 107 nestlings from 77 nests (Cíes: 34 nests ($n = 46$ chicks); Ons: 35 ($n = 51$ chicks) and Sagres: 8 ($n = 10$ chicks)) were blood sampled and several morphometries, including bill, wing and tarsus length, were measured (electronic supplementary material). Blood samples were stored in the cool until they were centrifuged on the same day, and plasma samples were stored frozen at -80°C. Each nestling was marked with a numbered metal ring and a coloured plastic ring with an individual two-digit combination to facilitate identification from a distance.

We determined the level of plasma antioxidant capacity for each sample by Trolox equivalent method (see the electronic supplementary material). The level of lipid peroxidation in plasma (i.e. oxidative damage in lipids) was assessed by quantifying malondialdehydes using high-performance liquid chromatography (see Noguera *et al.* [17] and the electronic supplementary material).

During the period 2007–2010, we collected resighting data through intensive field monitoring (all colonies were visited three to five times per year) of marked shags in the Parque Nacional das Illas Atlánticas. European shags with colour rings are easily detected and have a high probability of being resighted (91%, with 95% confidence interval 0.83–0.99; [18]). Juvenile shags are philopatric, and most breeders (greater than 99%) recruit to the island where they hatched [18]. We also consulted other resighting schemes from elsewhere in Spain, with more than 1000 resightings of shags between 2007 and 2010, but none of the study birds recruited to a site other than the natal colony. Average age of first reproduction in our study population is 2.53 years [18] and most shags recruit within age 3 years [19].

We evaluated variables affecting lipid peroxidation level (log transformed) during development using a linear mixed model (LMM) with nest identity and colony of origin as random terms (nest identity nested within the natal colony). In the model, sex (see the electronic supplementary material) was included as a fixed factor, and chick age, hatching date, brood size and chick body condition (see the electronic supplementary material) were included as covariates. Antioxidant compounds prevent oxidative damage [20], so we also included plasma antioxidant level as a covariate in the model. Recruitment probability was analysed using a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function. In this model, sex was included as a fixed factor, and lipid peroxidation, chick age, hatching date, brood size and body condition as covariates. Nest identity nested within the colony of origin was included as a random term. In 2009, invasive American minks (*Neovision vison*) were detected for the first time at shag breeding areas on Illas Cíes. Thus, to be confident that results were not affected by the presence of this exotic predator, we ran the same model but excluding this colony. In all models, Satterthwaite's approximation for degrees of freedom was used [21]. All models were simplified by removing non-significant terms (backward deletion) and the significance level was set at 0.05.

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Table 1. Summary of the minimal adequate models. (Model 1: LMM on lipid peroxidation and model 2: GLMM on recruitment of European shag chicks.)

dependent variable	independent variables	estimate (±s.e.)	F	d.f.	p
model 1					
lipid peroxidation	intercept	0.04554 (0.004)			
	plasma antioxidant capacity	−0.00326 (0.001)	5.88	1,102	0.017
model 2					
recruitment	intercept	0.5095 (0.297)			
	lipid peroxidation	−121.30 (42.802)	8.03	1,98.2	0.005

3. RESULTS

Mixed model analysis revealed that plasma antioxidant capacity was negatively related to lipid peroxidation in shag nestlings (table 1); plasma antioxidants explained 7.62 per cent of the variation in lipid peroxidation levels (figure 1). Sex, age, hatching date, brood size and body condition did not affect lipid peroxidation level ($p > 0.23$ in all cases), so they were excluded in the final model.

A total of 11 nestlings out of 107 recruited into the breeding population (5, 5 and 1 in Sagres, Ons and Cíes, respectively). GLMM on recruitment revealed that lipid peroxidation level was the only variable that significantly predicted recruitment: recruitment probability decreased as nestling oxidative damage increased (table 1 and the electronic supplementary material). On average, recruiting shags had lower level of lipid peroxidation as nestlings (1.074 ± 0.007 μg ml^{-1}) than non-recruits (1.085 ± 0.004 μg ml^{-1}). Similar results were achieved when the analysis was restricted to predator-free colonies (lipid peroxidation: $F_{1,51.11} = 4.93$, $p = 0.030$). Recruitment probability was not affected by sex, age, hatching date, brood size and body condition during the nestling period ($p > 0.10$ in all cases), so they were excluded in the final model.

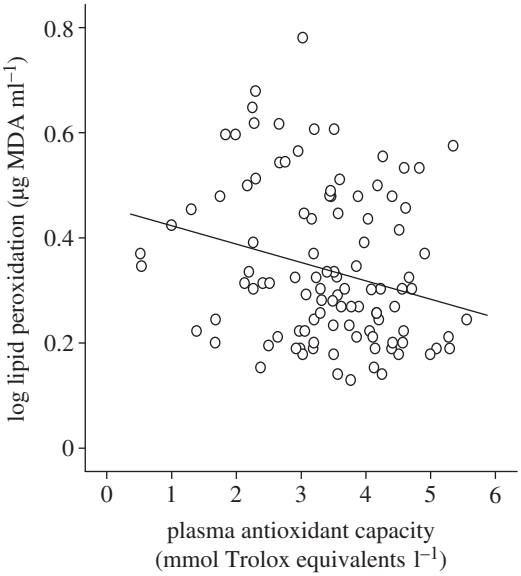

Figure 1. Relationship between plasma antioxidant capacity and lipid peroxidation (malondialdehyde, MDA) levels in European shag chicks (solid line shows a linear regression).

4. DISCUSSION

In this study, we found that shag nestlings with stronger antioxidant status had lower levels of lipid peroxidation, suggesting a link between antioxidants and oxidative damage during development. Most importantly, recruitment probability correlated with oxidative damage of nestlings. Because recruitment is closely related to post-fledgling survival in this study population [18], our results suggest a link between oxidative stress during development and survival to adulthood in a natural population of a long-lived bird.

Previous studies have shown that elevated growth rates may increase oxidative stress levels during early life [10,12]. Nevertheless, in our study, body condition, sex, hatching date and brood size (variables affecting growth rates [18]) did not influence lipid peroxidation in full-grown nestlings. Shag chicks with higher antioxidant capacity also had a lower level of lipid peroxidation, suggesting that availability of antioxidant reserves could play an important role in preventing oxidative damage during early development [20]. Shag nestlings often face unfavourable conditions during growth owing to, for example, parasitism [22], food shortage and cold stress during adverse weather ([23]; shags have delayed homeothermy [15]). Oxidative damage during development can be a consequence of stressful conditions, such as pollutants [24], thermal stress [25], compensatory growth [10] and nutritional deficiencies and diseases [26].

In our study population, most chick mortality occurs during the first two weeks after hatching [23], and natal dispersal to other colonies is very rare ([18]; in this study, all birds recruited to the natal colony). Although our analysis did not allow us to separate recruitment and survival, in our study site failure to be seen in the colony during the 3 years of life presumably reflects mortality during the first years after independence [18]. Our results highlight the possibility that lipid peroxidation level, during the nestling stage, may be a direct or indirect factor that anticipates future recruitment in seabird populations. Although no other predictor variable than lipid peroxidation was related to recruitment probability, we cannot exclude the possibility that this might be owing to the lack of statistical power.

In summary, our study provides empirical evidence for a link between oxidative damage during development and later performance in a vertebrate. This study suggests that oxidative stress (or related traits) may shape life-history traits of free-living organisms [27]. Future studies should experimentally address

whether factors governing oxidative status during the nestling period may have a delayed effect on recruitment and thereby on population dynamics.

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